CHAPTER - III

MATERIAL AND METHODS

A. MATERIAL

A. STUDY AREA

A.1. DIPLAI BEEL

A.1.i. Brief history of Diplai Beel:

The geographical coordinates of Diplai Beel are 26°16′5"N Latitude and 90°21′1"E Longitude. Its elevation is about 38.5 meter above mean sea level. It is the largest water body in Kokrajhar district covering an area of about 3.96 sq km. Its length and breadth are 3.30 km (North to South) and 1.20 km **(**East to West**)** respectively with a shoreline length of about 9 km. It is 15 km away from Kokrajhar town in the direction of South and can be reached by Silgara-Kokrajhar Road. Chakraborty, A.K. (2016) describes the history of Diplai Beel in his article '*Diplai Bilar eti Thulmul Bibaran'.* Source:'*Diplai Bilor eti Thulmul Bibaran'* written by Anupam Kumar Chakraborty, Head Master (retired), Silgara High School. Dist Kokrajhar, Assam.

Diplai Beel is situated in the Salkocha Mauza. Formerly it was under Koch King of Bijini State. Diplai Beel is surrounded by hills and hillocks and has an important history in this area. Its peak is called Dhola Pathor where it is believed that108 numbers of *pradip* like small holes are engraved over this big rock in ancient times. Every year in third day of Dol Purnima (Matia Holi), worship of Siva is performed by pouring fresh milk of 108 cows collected from the villages of this area. This festival is observed differently than others and has been popular in the district.

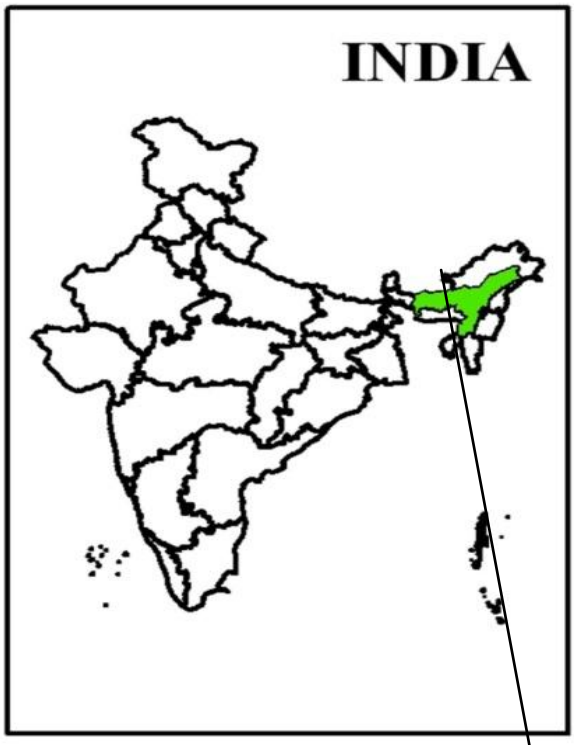
There is a belief that after pouring of milk on the Dhola Pathor it flows into Diplai Beel and the the meeting place is called Diplai Moth. People believe that in ancient times Lord Krishna appeared here in the shape of his four armed structure. Today the worship of Lord Krishna is being done in Pukhurpara Gosai Bari. It is also believed to be that there is a rock in the Chakrasila hill upon which Sudarshan Chakra of Lord Vishnu is engraved. There are still two rocks lying in the Joynagara village which are believed to be as Nagara (a big drum used in temple) and the echo of Nagara beating sometimes heard by villagers as they believe. Diplai beel was surrounded by dense forests in British India. Seeing its resources the son of Bijini

State King late Upen Kumar brought Desowali fishermen to catch fish to increase the treasury of the state by selling the fish to distant people of the country. After the abolition of Jamindari rule, the government turned this Beel into fish mahals. This beel is full of tales still.

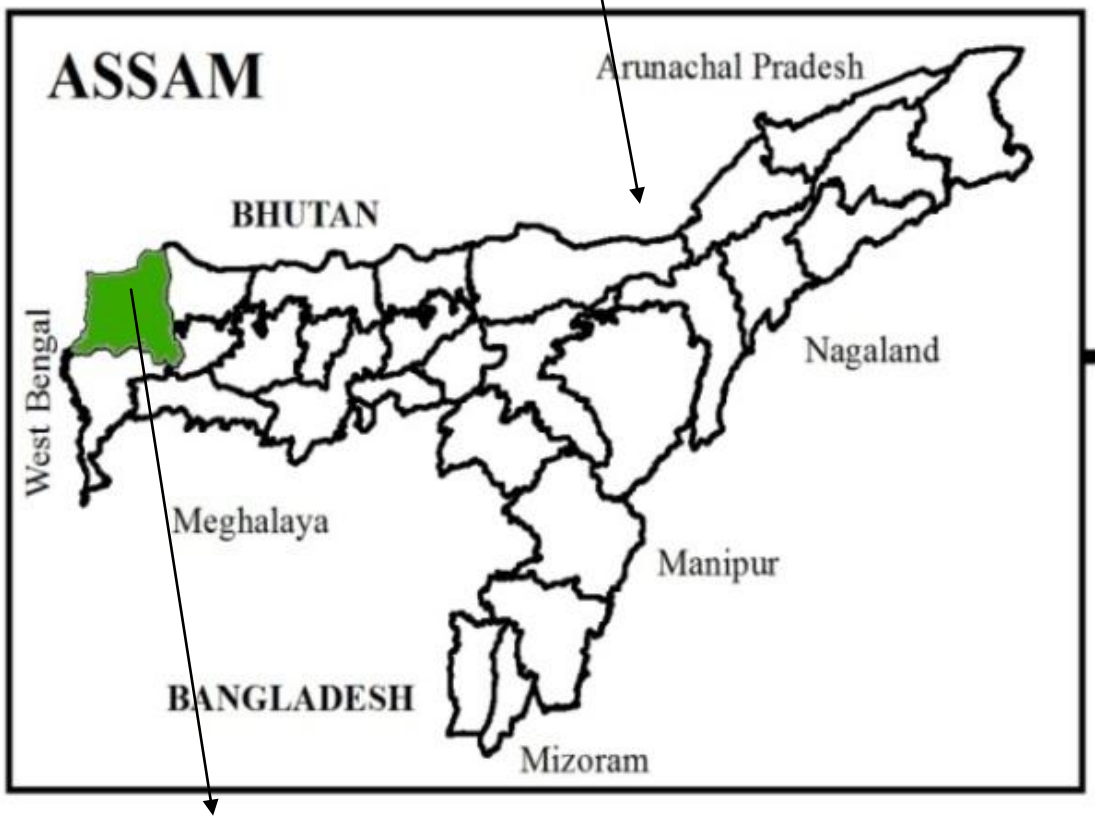
Diplai Beel is covered by moderately dense forests. In the East of Diplai beel there is a wild life sanctuary called Chakrasila wild life sanctuary covering 45.50 sq km in area. It has many endangered flora and fauna. Northern hill tracks are called Bou Kamari. The hilly tracts run north to south. Diplai Beel is an integral part of the whole eco-system. The lower hilly areas are covered with sal (*Soria rubasta*) trees while middle and upper parts are covered with mixed deciduous forests. Sal trees dominate the semi-evergreen and deciduous canopy of this sanctuary. The Beel has been turned to eco-tourism spot in recent times by providing accommodations to the tourists and travelers.

Diplai Beel is still a site of rich aquatic biodiversity and place of research for the scholars. It is a eutrophic beel due to abundant growth of hydrophytes. The shape of this water body is rectangular with few lobed projections and surrounded by hills in North (Hatimura and Chakrasila), West (Dholkoba), East (Batirmura and Jhoparia) and plain with few hillocks in South. The average depth of the beel in monsoon is 20 ft and in winter 10 ft approximately. It is connected to river Brahmaputra in the southern part and several streams flow down North to South are named as Sindurjhora, Borojhora and Bamuni but East to West is Hawhawijhora respectively. The area is associated with the process of erosion and siltation at present..

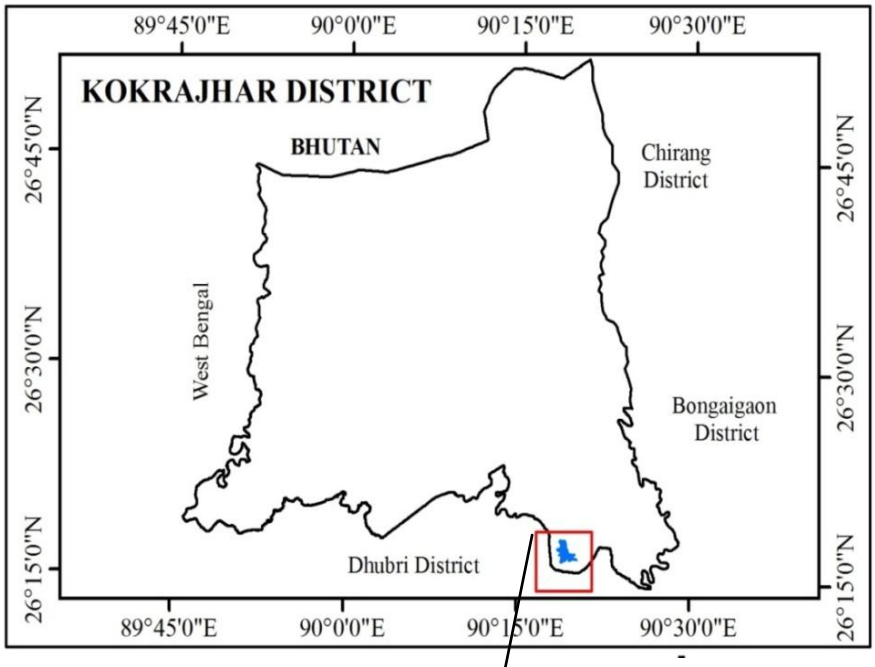
The surrounding hills are semi evergreen type. It is a source of green products for the forest department and the villagers of Diplai Beel area. It is surrounded by many revinew villages. In the South part of the beel the villages are Pukhuripara part-I,II,III and IV, Chotomalgaon-II and Supariguri, in the West Bethagaon, Bamunpara, Bamunpara-III, Barghola and Nalbari, in the North Daukibari part-I,II, Damodarpur-II,III, Bedlangmari and Harighola and in the East Abhayakuti, Salbari, Banapara, Jarnagra Part-I,III,IV. The density of population is less than other areas of Kokrajhar District. Villagers are totally dependent on agriculture and fishing. The catchment area of Diplai beel is encroached by the villagers for cultivation mainly paddy and thus the ecology of Diplai Beel has been a threat to nature lovers in recent times.



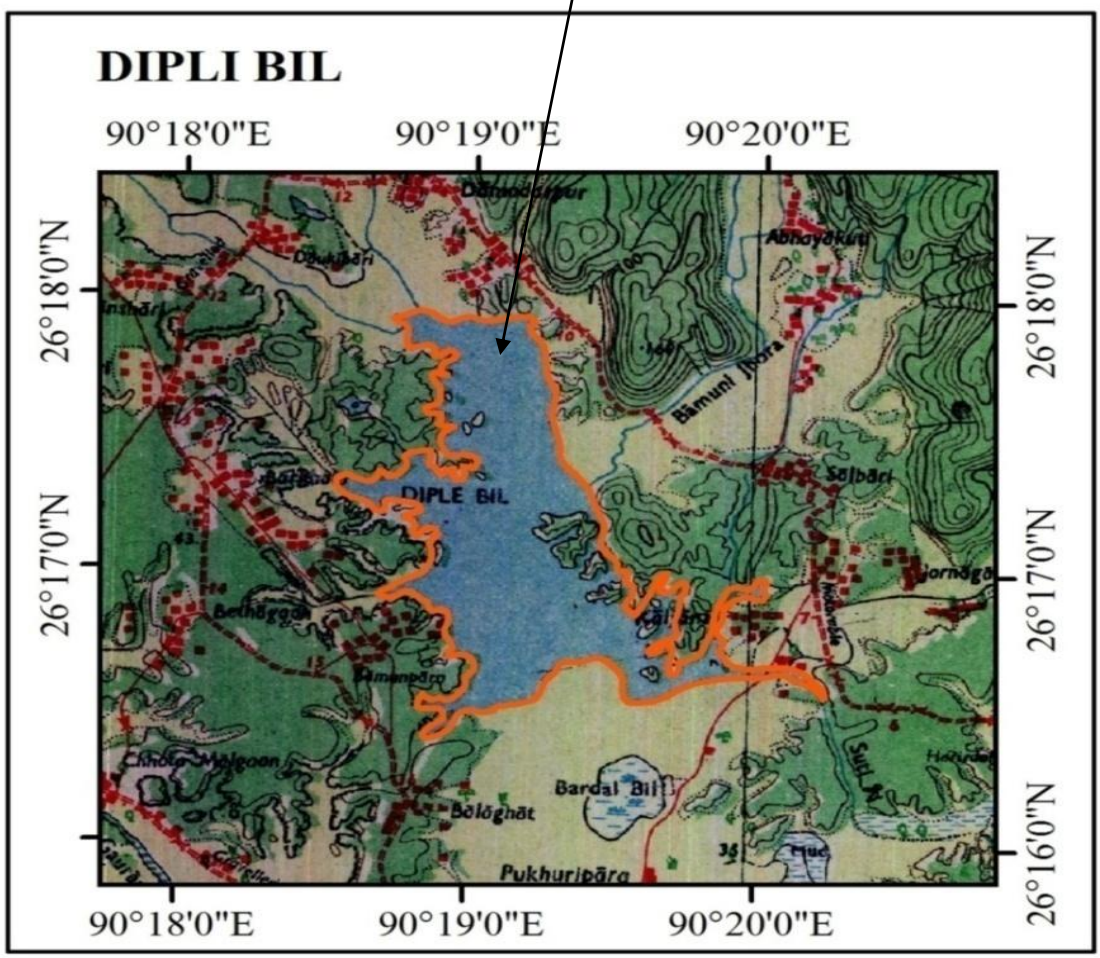
MAP OF INDIAN UNION and LOCATION OF ASSAM



Kokrajhar District **MAP OF ASAAM STATE**



Kokrajhar District and Location of Diplai Beel (Blue)



Satellite image of Diplai Beel (in Rainy season)

A.1.ii. Climate and Geography of Diplai Beel area:

Annual Rainfall	-	Max.-305 cm. Min.- 178 cm.
Average rainfall	-	211.76 cm.
Temperature	-	Summer-Max.33℃ , Min.17℃ Winter-Max.
		28 ℃ Min. 07 ℃
Average humidity	-	83.0%
Depth	-	Max- 21ft (monsoon),Min-12 ft (winter) (based on survey)
Weed coverage	-	Winter-20%, Summer- 95% (,,)
Catchment area	-	In winter- 2.63 sq Km (,,)
Encroachment	-	1.95 sq.km (,,)
Siltation	-	0.01 mm/yr (,,)

Diplai beel has perennial water and spread over an area of about 3.1 sq. km. which extends up to 6.1 sq. km. during heavy rain in rainy season. Generally the temperature varies from 10.6^0 to 32^0C. During winter months water area reduces by about fifty percent. In catchment area paddy is cultivated when the climate becomes relatively cool and dry. The tropical monsoon climate prolongs from May to September when it is humid. Pre-monsoon showers are seen during March to May.

Satellite view of Diplai Beel (Winter) (Source: Mapdata@2017. INEGI Imagery@2017NASA,Terra Matrics)

Satellite image of Diplai beel (black, white and green patches are water, cultivating land and hill & hillocks respectively)

B. METHODOLOGY

B. i. Design of Questionnaire:

A questionnaire headed by ***'Diplai Beel Research Questionnaire, 2014 to the villagers living around Diplai Beel and changes observed by them in last 20 years'*** is designed before writing the synopsis of my research work in 2013-14 to gather information of past and present ecological, biological, physico-chemical, resource utilization, cultural and socio-economic status of Dipai Beel. The information is collected from both the educated and uneducated villagers living around the water body. The individuals are interviewed and their responses to the questions asked in the ***Questionnaire*** are recorded for analysis (see appendix no.-1).

B. ii. Survey for selection of sampling sites:

Before selection of the sample collection sites a survey trip is done in the Diplai beel on a boat in 2014. The sites are then selected by fixing bamboo poles with flags basing on depths and water availability and named as follows.

i. **Site-I, (North)**
ii. **Site-II, (South)**
iii. **Site-III, (East)**
iv. **Site-IV, (West)**

B. iii. Seasons of sample collections in a year:

The sample collection period in a year is divided into four parts and records are maintained for three years from ***November 2014-1015 to November 2016-2017***

1. Pre-Monsoon (March, April and May)
2. Monsoon (June, July and August)
3. Post-Monsoon (September, October and November)
4. Winter (December, January and February)

C. METHODS OF MACROPHYTE STUDY:

C. i. Macrophyte Collection:

Quadrat method of *Cain and Castro (1959)*

Before the start of sample collection some preparation must be taken by surveyor or collector such as first aid box, pen, pencil, exercise book, plastic bottles, polythene bags, knife, blotting paper, hook, bamboo sticks, preservatives etc. *Quadrat*

method of Cain and Castro (1959) is followed here. A quadrat of size 2 sq.m. is made by PVC pipes with PVC angle joints. In 2014-2015, 2015-2016 and 2016-2017 every month 10 quadrats readings are taken randomly in four selected sites namely Site-I, (North), Site-II, (South), Site-III, (East), Site-IV, (West) in the Beel which represent opposite to each other. The types of macrophytes collected are **i. Free Floating, ii. Submersed-(anchored), iii. Submersed-(suspended), iv, Rooted Floating Shoot, v. Rooted Floating Leaves and vi. Emergents.** Plants are collected by free hand and sometimes by hooks or by nets of different sizes of meshes from over a boat. Date, site name, weather and time of collection etc. are recorded in log book with collection numbers and plants are placed inside the polythene bags with little water. The small macrophytes are put inside bottles in water with preservatives then and there. After collection all species are brought in the laboratory.

C. ii. Handling and Preservation of collected macrophyte species:

Macrophytes thus collected are pressed in absorbents such as blotting papers or news papers under different degree of pressures at regular intervals. Then the dried specimens are poisoned by spraying Kew mixture (115 gm of chloride dissolved in 4.5 litres of ethyl alcohol). After poisoning the specimens are dried again in air and affixed by fevicol gum over herbarium sheets of size 28 cm $\times$42cm $\pm$1 cm each giving labels of serial numbers with threads. After this the process of scientific identification is done in laboratory.

C. iii. Identification of macrophytes:

The collected specimens are identified referring the 'Flora of British India' (Vol.1-7) by Hooker (1872-1897); 'Flora of Assam' (Vol.1-4) by Kanjilal *et al.* (1934-40): 'A Manual of Aquatic Plants' by Fasset (2000); 'Water plants of the world' by Cook (1974) ; Cook (1996) ; 'A hand book of field herbarium methods' by Jain *et al.* (1978); 'Weedy aquatic plants their utility, menace and management' by Gupta (2001). Herbaria, Department of Botany, GU, Guwahati; Herbaria of BSI, Shillong.

C. iv. Study of Phytosociological characters of macrophyte species in Diplai Beel:

Calculation of Diversity Indices (Species richness, Evenness) Density,

Abundance and IVI:

To study the phytosociological characters of the Diplai Beel, the quadrat of 2 m × 2 m size is used within the plants community.

The diversity indices like (1). Species richness (Margalef, 1964), (2). Shannon-Weaver, (3). Diversity Index (Shannon and Weaver, 1963), (4). Simpson Dominance Index (Simpson, 1949), and (5). Species evenness index (Pielou,1966) are also calculated.

C.iv.a. Species richness (d): $d = S/\sqrt{N}$

Where, S=Total number of species, and

N =Total number of individuals of all the species.

C.iv.b. Shannon–Weaver index of diversity (**H´**):

$$H' = -\Sigma\, pi \ln pi$$

Where, pi = the proportion of Importance Value of the Ith specie, pi = ni / N, (ni is the Importance Value of ith species) and N = Importance Value of all the species).

C.iv.c. Simpson's index of Dominance (D): $D = \Sigma (pi)\, 2$

Where, pi = the proportion of Important Value of the ith species (pi = ni / N, ni is the Importance Value of i th species and N = Importance Value of all the species).

C.iv.d. Evenness index (**E**): $E = H'/\log S$

H´= Shannon–Weaver diversity log S= Natural log of the total number of species recorded.

C.iv.e. Density (**D**) **D =** Total no of individual Species / Total no. of quadrats studied per sq m

C.iv.f. Abundance (Ab) Ab = Total no of individual species / No of Quadrates plants occurrence

The phytosociological characteristics such as Density, Frequency, Abundance and their relative values are calculated by using the methods of Misra (1969). The Importance Value Index (IVI) of the plant species is also calculated by the method of Curtis (1959) and Devi (1993).

C. iv.g. Density

Density is an expression of the numerical strength of a species per unit area where the total number of individuals of each species in all the quadrats is divided by the total number of quadrats studied. Density is calculated by method of Misra (1969).

Density = Total number of individuals of the species in all quadrats is divided by Total number of quadrats studied

C.iv.h. Relative Density

Relative density is the study of numerical strength of a species in relation to the total number of individuals of all the species and it is measured by the formula of Misra (1969).

Relative Density = Density of the species × 100 is divided by Density of all the Species

C.iv.i. Frequency (expressed in %)

Frequency is defined as the chance or probability of an individual of a given species to be present in a randomly placed quadrat. Frequency is calculated by the formula of Misra (1969). It is the degree of dispersion of individual species in an area and expressed in percentage. It is studied by sampling the study area at several places at random and to cover the site adequately and recorded the names of the species that occurred in each sampling units.

Frequency (%) = Number of quadrats in which the species occurred × 100 is divided by Total number of quadrats studied.

C.iv.j. Relative Frequency

The degree of dispersion of individual species of an area in relation to the number of all the species occurred. It s calculated by the method of Misra (1969).

Relative frequency = Frequency of the species × 100 is divided by Frequency of all the species.

C.iv.k. Abundance

It is the number of individuals of different species in the community per unit area. Abundance is measured by the method of Misra (1969). In this method samples are made randomly at several places and the number of individuals of each species is added of all the quadrats and is divided by the total number of quadrats in which the species occurred. Abundance do not ventilate a total picture of numerical strength of a species in an area because only the quadrates of occurrence are taken into consideration and not all the quadrates studied.

Abundance = Total number of individuals of a species in all quadrats, is divided by Total number of quadrats in which the species occurred.

C.iv.l. Relative Abundance

It is the study of the number of individuals of different species in a community per unit area.

Relative abundance = Abundance of the species×100 is divided by Abundance of all the species

C.iv.m. Importance Value Index (IVI)

Importance Value Index is used to determine the overall importance of each species in the community. It is expressed out of 300. This value is designated as the Importance Value Index or IVI of the species (Curtis, 1959).

It is observed that due to the differential growth forms of water macrophytes such as free floating, submerged, suspended, rooted with floating leaf/ stem, emergent plant species etc, it is very inconvenient to estimate the relative dominance of the species. It is also very difficult to determine the basal coverage area of the macrophytes in water surface as the substrate area.

The determination of the basal area of the aquatic macrophytes due to having different growth forms is faced by plenty of problems and anomalies for calculation of dominance of aquatic macrophytes (Devi, 1993). *Therefore, it is considered as Relative Abundance instead of Relative Dominance to overcome the problems.* The IVI is calculated by addition of the percentage values of Relative Density (RD), Relative Frequency (RF) and Relative Abundance (RA).

Importance Value Index (IVI) = RD + RF + RA

D. METHODS OF WATER ANALYSIS:

D. i. Water sample collection:

A sample is a representative of a matter where all relative proportions or concentration of all pertinent components will be of same nature. Water samples are collected monthly in1L PVC bottles in between 9 A.M. to 11 A.M. at a depth of 5 cm to 8 cm from four selected spots of Diplai Beel. Before collection, the water sample bottles are rinsed and washed twice or thrice. The bottle mouth is then inclined to 45˚ and water is collected from the selected spots. The bottle is tighten with the lid and a

label is pasted on bottles for date, code no, time, location etc. The odour, colour, water Temperature, pH, DO, BOD, Electrical Conductivity (EC), Turbidity, Total Dissolved Solids (TDS) are determined in the spots of collection and the others are analyzed in the laboratory within 72 hours. For DO, DO bottle is used to collect water.

D. ii. Sample handling and Preservation:

Proper emphasis is given after sample collection because the quality of the water sample would change due to physical, chemical and biochemical reactions. So to reduce these changes in the water sample, it is necessary to preserve the water sample as early as possible soon after collection. By adding chemical preservatives the water samples are preserved. The temperature of water and atmosphere, pH, Dissolved Oxygen, EC and Total Dissolve Solids are analyzed immediately during collection period on the spots, while the remaining parameters are analyzed in the laboratory through proper preservation. The different preservation methods are used for the different water parameters and their methods of analysis are shown separately with each parameter. (Methods of BIS and APHA followed)

D.iii. WATER ANALYSIS:

No.1 Water Temperature: C°

The surface water temperature is measured by a Mercury Thermometer of 0ºC to 100ºC range. The thermometer bulb is dipped in the surface water of Diplai Beel up to 4 cm to 8 cm and kept for 1 to 2 min then temperature is recorded immediately in log book. It is repeated twice or thrice in the site for confirmation.

No.2 Air temperature: C°

The atmospheric temperature is measured with the help of Max. and Min. thermometer in open air near the Diplai Beel.

No.3 Colour (HU)

Method name: - Platinum cobalt (Visual Comparison) method

Specification of the method: - *IS 3025 (part4)-1983, Reaff 2002.*

Equipment used: - Nessler cylinder 50ml capacity, filter assembly

Ref. standard used: - K_2PtCl_6

Environmental condition: i. Required for stabilization / storage - 4ºC.

ii. Required for test- Room temperature

Principle:

Colour is determined by visual comparison of the sample with known concentration of the colour solution. Comparison also may be made with special, properly calibrated glass colour disks.

Reagent:

i. Dissolve 1.246 g K_2PtCl_6 and 1g cobaltous chloride in distilled water with 100 ml concentrated HCl and diluted to 1000 ml with distilled water. The stock standard has a colour of 500 units.

ii. Prepare a series of std.s having colours of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70 by diluting 0.5, 1.0, 1.5, 2.0, 2.5, 3, 3.5, 4, 4.5, 6 and 7 ml stock colour std. with distilled water to 500 ml in Nessler tubes. Use distilled water as 0 standards.

Procedure:

Sample showing visible turbidity should be clarified by centrifugation or filter assembly with glass fiber filters. Observe sample colour by filling matched a Nessler tube to 50 ml mark with Sample and comparing it with the standards prepared. Compare by looking vertically downwards through the cylinders towards a white surface placed at such an angle that light is reflected upwards through the column of liquid.

Calculation

Colour unit = A x 50/V

Where, A = estimated colour of a dilute sample

V = ml sample taken for dilution

Report:

Colour Units	Report to nearest
1 to 50	1
51 to 100	5
101 to 250	10
251 to 500	20

No.4 Odour agree/disagree

Method name: Platinum cobalt (Visual Comparison) method

Specification of the method: - ***IS 3025 (part5)-1983, Reaff 2002.***

Equipment used: - Glassware

Ref. standard used: - Odour free water

Environmental condition: - i) Required for stabilization/storage- Not applicable.

ii) Required for test- Room temperature

Principle:

Odour is detected when the odour causing substance is volatile and is released as a vapour from the sample therefore the degree to which odour is detected depends on the vapour pressure and solubility of the substance in the sample.

Reagent:

i. HCl
ii. Odour free distilled water.

Preparation of Apparatus:

First of all clean a white mouth glass stoppered bottle of 1 liter capacity with HCl repeatedly to make completely odourless and then wash repeatedly with odour-free distilled water.

Procedure:

As soon as possible after collection of sample, fill a bottle half full of sample, insert the stopper and shake for 2-3 sec. and then quickly observe the odour. The sample taken for observation shall be at room temp. When it is desired to record the odour at an elevated temperature, make the observation after warming the sample in a clean stopper bottle to about 60^0C.

Report:

Report the true odour of the sample at the mouth of the bottle as rotten egg, burnt sugar, and soapy, fishy, septic, aromatic, chlorinous, alcoholic odour. In case it is not possible to specify the exact nature of odour, as agreeable or not agreeable**.** In case of waste water odour shall be described as the degree of sweetness, pungency, smokiness and rottenness. If the characteristic being judged is high in intensity, rate that characteristic as 100, if medium rate as 50, if low rate as 0**.**

No. 5 Total Suspended Solids (TSS) mg/L

Specification of the method: - ***APHA 2540 D***

Equipment used: -

a) High silica glass for evaporation
b) Steam bath

c) Desiccator
d) Drying oven
e) Analytical balance.
f) Magnetic stirrer
g) Filtration assembly
h) Filter paper

Principle:

A well mixed sample is filtered through a weighed standard glass fibre filter and the residue retained on the filter is dried to a constant weight at 103°C to 105°C. The increase in weight of the filter represents the suspended solids. If the suspended material clogs the filter and prolongs filtration, it may be necessary to increase the diameter of the filter or decrease the sample volume. To obtain an estimate of total suspended solids, calculate the difference between total dissolved solids and total solids.

Procedure:

i) Preparation of evaporating dish: Wash the dish or beaker with reagent grade water and dry in an oven at 103°C to 105°C for 1 hr until a const weight is found. Store and cool the dish or beaker in desiccators until needed. Take weight immediately before use.

ii) A filter paper is placed in a filter assembly and wet with reagent grade water. 20 ml of three successive portion of reagent grade water is allowed to pass through the filter paper using vacuum pump to remove all traces of water. Vacuum is turned off and washings are discarded. The filter paper is removed from the assembly and with a support of a disk it is dried at 103 °C to 105°C for 1 hr. After cooling in desiccators the filter is weighed to get a constant weight.

iii) Selection of filter and sample size: Choose sample volume to yield between 2.5 and 200 mg dried residue. If volume filtered fails to meet minimum yield, increase sample volume up to 1 ltr. If complete filtration take more than 10 min, increase filter diameter or decrease sample volume.

iv) Filter paper is placed in the filtration assembly and a measured volume of homogenous sample is allowed to pass through the filter paper and then wash the filter with three successive 10 ml volumes of reagent grade water with continue suction. Filter paper is removed from the assembly and

transfer to the supporting disk and dried at 103°C to 105°C, cooled in desiccators and finally weighed to get a constant value.

Calculation:

Total suspended solids/L= (A - B) × 1000 mg Sample volume, ml

Where A= weight of filter + dried residue, mg

B= weight of filter, mg

No.6 Total Dissolved Solid (TDS) mg/L

Method name: **Gravimetric method**

Specification of the method: - ***IS 3025 (part 16)-1984***

Equipment used: - Analytical balance (0.1mg-200g), membrane filter (0.45µ membrane),dry oven, desiccators

Ref. standard used: - No need

Environmental condition: - i. Required for stabilization / storage- 4ºC

ii. Required for test: - Room temperature

Principle:

The sample is filtered and the filtrate evaporated in a tarred dish on steam bath. The residue after evaporation is dried to constant mass at 103-105˚c or 179°C - 181˚C.

Procedure:

Heat a clean evaporating dish to 180˚C for 1 hr. Cool it in the desiccators, weigh and store until ready to use. Filter a portion of sample using filtering assembly. Select a volume containing residue preferably between 100 to 200 mg and is pipetted to the weighed evaporating dish placed on a steam-bath or in a drying oven (approx at 98˚C). After complete evaporation the residue containing dish is transferred to an oven at 180˚C and dry to constant mass for 1 hr. cool the residue containing dish in the desiccators and weigh.

Calculation: T.D.S. (mg/l)=1000 M / V, at 180˚C.

Where, M= mass in mg of filterable residue

V= volume in ml of sample.

No.7 Turbidity NTU

Method name: - Nephelometric Turbidity method

Specification of the test: - ***IS 3025 (part10)-1984, Reaff 2002.***

Equipment used: - Nephelometric turbidity meter

Reference standard used: - Standard turbid solution

Environmental condition:- i. Required for stabilization/storage: Refrigeration to 4ºC. ii. Required for test :- Room temperature

Principle:

It is based on comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a reference suspension under the same conditions. The higher is the intensity of scattered light, the higher the turbidity.

Reagents:

i. Distilled water

ii. Hydrazine sulphate [$(NH_2)_2.H_2SO_4$]-1% Aqueous solution……. solution 1(w/v)

iii. Hexamethilene Tetramine $(CH2)_6N_4$ 10% Aqueous solution……..…… solution 2(w/v)

iv. Turbid standard suspension I (400 NTU)- In a 100ml flask mix 5ml solution b) and 5 ml solution c) Let it stand for 24 hours at 25± 3˚C.Dilute to 100ml with distilled water and mix well. This results in a 400 NTU suspension. Dilute the standard to 1, 2, 5, 10, 20, 40 NTU solution.

v. Turbid standard suspension II- 10 ml turbid standard suspension I is diluted to 100 ml = 40 NTU solution.

Procedure:

i. Calibration of instrument:

Turn the instrument on. Set range at 0–100. Place a round cub with distilled water into the instrument so that the markings of the instrument and the round cub coincide with each other. Set zero by moving zero adjustment knobs. Place a round cub with 40NTU Standard turbid solution in the instrument and set indicator needle at 40 by moving 'CALIB' knob. Again place round cub with distilled water to check zero.

ii. Turbidity less than 40 NTU:

Shake the sample to disperse the solids and wait until air bubble disappear and pour sample into cell read turbidity directly from instrument.

iii. Turbidity greater than 40 Units:

In case turbidity value greater than 40 Units, dilute the sample with turbidity free water to bring the value within range.

Calculation: For diluted sample

Turbidity units=A× (B+C)/C

Where A= Turbidity units found in diluted sample,

B= Volume in ml of dilution water used,

C= Volume of sample in ml taken for dilution.

Report turbidity reading as follows:

Turbidity Range NTU	Report to the Nearest
0 – 1.0	0.05
1 – 10	0.1
10 – 40	1
40 – 100	5
100 – 400	10
400 – 1000	50
> 1000	100

No. 8 Transparency Secchi Disc (m)

Principle

The light attenuation coefficient, k, can then be used in a form of the **Beer–Lambert law**, to estimate I_z, the intensity of light at depth z from I_0, the intensity of light at the ocean surface. **Iz/Io=e**-kz

Method:

The Secchi disk, created by Angelo Secchi in 1865, is a plain white, circular disk 30 cm (12 in) in diameter. It is used to measure water transparency of water. The depth at which the disk is no longer visible is taken as a measure of the transparency of the water. The period for best results is between 10:00 and 14:00 of a sunny day. The depth at which the disk disappears and lower again another few feet and then the depth is recorded at which the disk reappears when it is slowly brought up. The Secchi disk depth is taken as the average of the two values.

No. 9 pH

Method name: Electrometric method is applicable to all types of water

Specification of the Test: - ***IS-3025(part11)-1983, Reaff 2002.***

Name of the method: - Electrometric method

Equipment used: - pH meter, thermometer with least count 0.5˚C

Ref. standard used: - NIST standard buffer solutions of pH 4.0, 7.0 & 9.0

Environmental condition:- i) Required for stabilization / storage: - Not applicable.

ii) Required for test: - Room temperature

Principle:

The pH value is determined by measurement of the electromotive force of a cell consisting of an indicator electrode (an electrode responsive to hydrogen ions such as a glass electrode) immersed in the test solutions and a reference electrode (usually mercury/calomel electrode). Contact between the test solution and the reference electrode is usually achieved by means of a liquid junction, which forms part of the reference electrode. The electromotive force is measured with a pH meter, i.e., a high impedance voltmeter calibrated in terms of pH.

Reagents:

Preparation of standard buffer solutions:-

i. Buffer 4.0
ii. Buffer 7.0
iii. Buffer 9.2

Procedure:

Rinse the glass electrode with distilled water and gently wipe it with a soft filter paper. The determination may now be made by pH meter with glass electrode at room temperature or at temperature as per sample requirement after standardizing the pH meter with buffers 4.0, 7.0 and 9.2 and directly reading the digital pH reading.

Calculation:

Read pH directly from instrument.

No.10 Electrical conductance: μS/cm

Principle:

The resistance of the solution (R) can be calculated using Ohm's law ($V = R \times I$). Conductance (G) is defined as the reciprocal of the electrical resistance (R) of a solution between two electrodes. The conductivity meter in fact measures the conductance, and displays the reading converted into conductivity.

Method:

Electrical Conductivity (EC) is measured by digital EC meter, HM digital, (model COM-100). Cap of the Conductivity Meter is removed and pressed the switch on. Display will become active. The default mode is for EC in the meter µS/cm scale. It is adjusted. Dip the meter in water. After few seconds meter will display a reading until reading is stabilized. Reading is recorded and meter is made off.

No.11 Alkalinity mg/L

Method Name: Indicator method. Potentiometric method is applicable for determination of alkalinity in water and waste water in the range 0.5 to 500 mg/l as $CaCO_3$

Item to be analyzed: - Water / package drinking water

Specification of the method: - *IS-3025(part23)-1986, reaff 2003*

Equipment used: - Burette, magnetic stirrer, pH meter.

Ref. standard used: - NIST standard Na_2CO_3

Environmental condition: - Required for test: - Room temperature

Principle:

Alkalinity of water is the capacity of water to accept protons. It is also defined as the capacity of an aqueous medium to react with hydrogen ions to pH 8.3 (phenolphthalein alkalinity) and then to pH 3.7(methyl orange alkalinity).

$CO_3^{2-} + H^+ = HCO_3^-$ (pH 8.3), $HCO_3^{1-} + H^+ = H_2CO_3$ (pH 3.7)

Reagents:

i. Sulphuric acid: Dilute 5.6 ml of Conc. H2SO4 (relative density 1.84) + D/W up to 1 litre.

ii. 0.02(N) H_2SO_4

iii. Mixed indicator solution: Dissolve 0.01g bromocresol green and 0.02g methyl red in100ml 95% ethyl alcohol or isopropyl alcohol.

iv. Phenolphthalein solution alcoholic : Dissolve 0.5 gm Phenolphthalein in 100ml 1:1alcohol water mixture

Procedure:

If sample is turbid then make it free from turbidity. Take 50 ml sample or as required. If pH of sample exceeds 8.3 then, add 2-3 drops of phenolphthalein indicator and titrate with 0.02(N) H_2SO_4 till the pink colour observed by the indicator just

disappears (pH 8.3) and record the volume of H_2SO_4 solution used. Add 2 to 3 drops of mixed indicator to the solution in which the phenolphthalein alkalinity has been determined. Titrate with the standard acid to light pink colour (pH 3.7). Record the volume of standard acid used after phenolphthalein alkalinity.

Calculation:

Total Alkalinity (mg/l) = (A+B) X N X 50000) / ml of sample

where, A= ml of standard sulphuric acid used to titrate to pH 8.3

B= ml of standard sulphuric acid used to titrate from pH 8.3 to pH 3.7

N = Normality of H_2SO_4

No.12 Biochemical Oxygen Demand (BOD) mg/L

Method Name:- Oxygen depletion method based on bioassay procedure for measurement of biochemical oxygen demand.(5 days method

Specification of the method: - ***IS 3025 (part 44) : 1993***

Equipment used : - i) Incubation bottle ii) Incubator ii) Glassware

Environmental condition: i) Required for stabilization / storage: - 4 ºC.

ii) Required for test: - Room temperature

Principle:

The BOD test is based on mainly bio-assay procedure which measures the dissolve oxygen consumed by micro-organisms while assimilating and oxidizing the organic matter under aerobic conditions.

Reagent:

i. Phosphate buffer solution:

Dissolve 8.5 g potassium dihydrogen phosphate, 21.75 g potassium Hydrogen phosphate, 33.4 g disodium hydrogen phosphate and 1.7 g ammonium chloride in about 500 ml distilled water and dilute to 1 ltr. pH of the solution should be 7.2 without any adjustment.

ii Magnesium sulphate solution:

Dissolve 22.5 g magnesium sulphate in distilled water and dilute to 1 ltr.

iii. Calcium chloride solution:

Dissolve 27.5 g calcium chloride in distilled water and dilute to 1 ltr.

iv. Ferric chloride solution:

Dissolve 0.25 g hydrated ferric chloride in distilled water and dilute to 1

ltr.1 N sodium hydroxide and 1 N sulphuric acid for neutralization of samples.

v. Glucose-glutamic acid solution:

Dry reagent grade glucose and reagent grade glutamic acid at 103°C for 1 hr.Add 150 mg of glucose and 150 mg of glutamic acid to distilled water and dilute to 1 ltr. Prepare fresh immediately before use.

Procedure:

i. Preparation of dilution water: Prepare dilution water by adding 1 ml phosphate buffer solution, 1 ml magnesium sulphate solution, 1 ml calcium chloride, 1 ml ferric chloride solution in 1 ltr.

ii. Add 2 to 5 ml of treated sewase water per ltr of dilution water for seeding purpose.

iii. Prepare the desired percentage mixture by adding sample in dilution water.

In case of high BOD value:

a. Take required quantity of sample in a 1 ltr volumetric flask and volume make up to the mark with the diluted water and mix. Rinse two bottle with the mixture and fill them with the mixture up to over flow and then go for incubation to one of them. Determine the DO of 1 bottle immediately and other after 3 days incubation at 27±1°C.

b. Fill up 1 bottle with the dilution water blank and determine the DO value after 3 days incubation at 27±1°C .

In case of BOD less than 5 mg/l:

Samples of natural surface water bodies like river, lake and marine, generally do not require seeding and dilution due to naturally available microbiological population and low BOD value. For such sample take 100 % pure sample i.e without any dilution.

Calculation:

i) When sample is undiluted BOD, mg/l= DO before incubation-DO after incubation

ii) When dilution water is unseeded BOD, mg/l = D1 – D2 × 1000 P

iii) When dilution water is seeded BOD, mg/l = D1-D2-(B1-B2) f × 1000 P

D1=Initial DO of sample in mg/l

D2= DO of sample after incubation in mg/l

P = % dilution mixture = sample add ×100% = sample vol. in ml1000 10

B1= DO of seed control before incubation in mg/l

B2 = DO of seed control after incubation in mg/l

F = Ratio of seed in diluted sample to seed in control i.e

= (Percent seed in diluted sample) : (Percent seed in seed control)

No.13 Chemical Oxygen Demand (COD) mg/L

Method name: Closed reflux method

Specification of the method: - ***IS 3025 (part 58): 2006.***

Equipment used: i) Digestion vessel

ii) Autoclave

Ref. standard used: Standard. Potassium dichromate

Environmental condition:

i) Required for stabilization / storage: - 4 ºC.

ii) Required for test: - Room temperature

Principle:

Most of the organic matters are destroyed when boiled with a mixture of potassium dichromate and sulphuric acid producing carbon dioxide and water. A sample is refluxed with a known amount of potassium dichromate in sulphuric acid medium and the excess of dichromate is titrated against ferrous ammonium sulphate. The amount of dichromate consumed is proportional to the oxygen required to oxidize the oxydizable organic matter.

Reagent:

i) Standard Potassium Dichromate 0.25 N : Dissolve 12.259 g Potassium dichromate previously dried at 150 for 2 h, in distilled water and diluted to 1 ltr.

ii) Sulphuric acid concentrate: Add 5.5 g silver sulphate to 1kg concentrated sulphuric acid bottle and let stand 1 to 2 d to dissolve and mix.

iii) Standard ferrous ammonium sulphate 0.25 M: Dissolve 98 g ferrous ammonium sulphate in distilled water. Add 20 ml conc. H_2SO_4, cool and diluted to 1 ltr.

iv) *Standardize this solution daily against standard K_2Cr2O_7 as follows:*

i. Dilute 25 ml Std. $K_2Cr_2O_7$ to about 100 ml. Add 30 ml conc. H_2SO_4 and cool. Titrate with FAS titrant using 2-3 drops ferroin indicator. Molarity of FAS solution = Vol. 0.04167 M $K_2Cr_2O_7$ solution titrated, ml × 0.25 Vol. FAS used in titration, ml

ii. Ferroin indicator: Dissolve 1.485 g 1, 10-phenanthroline monohydrate and 695 mg $FeSO_4.7\ H_2O$ in water and diluted to100 ml. The indicator solution may be purchased already prepared.

ii. Mercuric sulphate, $HgSO_4$ crystal

Procedure:

i. Place 5 ml or fraction diluted to 100 ml of sample with distilled water in hard glass bottle and add 25 ml standard potassium dichromate solution. Carefully add 75 ml conc. $H2SO_4$ mixing after each addition. Digest the mixture in autoclave for 30 min.

ii. Repeat the procedure with 100 ml distilled water and reagent as in (i).

iii. Transfer the content to a 500 ml conical flask. Dilute the mixture to about 350 ml. Titrate the excess dichromate with standard ferrous ammonium sulphate using ferroin indicator. The end point is red. Designate the titration value for sample (i) as B for distilled water (ii) as A.

Calculations:

COD mg/l = (A-B) C×8×1000 ml sample

where

A= ml $FeSO_4\ (NH_4)_2SO_4$ used for blank

B= ml $FeSO_4\ (NH_4)_2SO_4$ used for sample

C= Normality of $FeSO_4\ (NH_4)_2SO_4$ solution determined above.

14. Dissolved Oxygen (DO) mg/L

Method name: - Titrimetric Winkler's method

Specification of the method: - ***IS 3025 (part 38) : 1989***

Environmental condition:-

i) Required for stabilization / storage: - 4 ºC.

ii) Required for test: - Room temperature

Apparatus: - i) Glassware

ii) weighing balance

Principle:

Divalent manganese salt in solution is precipitated by strong alkali to divalent manganese hydroxide. It is rapidly oxidized by dissolved oxygen present in the sample to form trivalent or higher valency hydroxide. Iodide ions are added and acidified, which reduce tetravalent hydroxides back to their stable divalent state thereby liberating equivalent amount of iodine. This iodine is equivalent to dissolved oxygen present in the sample.

Reagent:

i) Manganous sulphate solution: Dissolve manganese sulphate (480g of $MnSO_4.4H_2O$ or 400 g of $MnSO_4.2H_2O$ or 364 g of $MnSO_4.H_2O$) in freshly boiled and cooled water, filter and make up to 1000 ml. The solution should not give blue colour by addition of acidified potassium iodide solution and starch.

ii) Alkaline iodide solution: Dissolve 500 g of sodium hydroxide (or 700 g potassium hydroxide) and135 g of sodium iodide or (150 g of potassium iodide) in freshly boiled and cooled water and diluted to 1 ltr.

iii) Sulphuric acid, concentrated.

iv) Starch indicator: Dissolve 2 gm starch and 0.2 gm salicylic acid as preservative, in 100 ml of hot distilled water.

v) Sodium thiosulphate stock solution: Dissolve approximately 25 g of sodium thiosulphate in boiled distilled water and make upto 1 ltr. Add 1 g of sodium hydroxide to preservative it.

vi) Standard sodium thiosulphate solution: Dissolve 250 ml of stock solution in boiled distill water and make up to 1 ltr and standardize sodiumthiosulphate against known standard before use.

Procedure:

To the sample collected in 300 ml bottle add 2 ml manganese sulphate solution+ 2 ml alkaline iodide solution then stopper the bottle and mix thoroughly, allow the precipitate formed to settle. After 2-3 mins of settling, carefully remove the stopper and immediately add 2 ml of conc. H_2SO_4 and mix thoroughly to dissolve the liberated iodine. Take 200 ml of the solution and titrate immediately against standard sodium thiosulphate solution, adding 3-4 drops of starch indicator solution. The end point is pale blue to colourless.

Calculation:

The dissolved oxygen in mg/l is equal to the volume in ml of 0.025 N Thiosulphate solution used for titration.

No.15 Sulphate as SO_4 mg/L

Method name: - Turbidity method

Specification of the method: - ***IS-3025(part-24)-1986, Reaff 1992***

Equipment: - Glassware,Spectrophotometer/nephelometric turbidity meter

Ref. standard used: - NIST std. sulphate solution

Environmental condition:-

i) Required for stabilization / storage :- 4ºC

ii) Required for test :- Room temperature

Principle:

Sulphate ion is precipitated in hydrochloric acid medium with barium chloride so as to form barium sulphate. The absorbance of barium sulphate suspension is measured by a spectrophotometer and the sulphate ion concentration is determined from standard curve.

Reagents:

Conditioning reagent:-

i. 0.3g gelatin in 100ml D/W and warm till dissolve. Keep it at 4˚C for 12hrs or overnight. After bringing the solution to room temperature, add 3g of barium chloride and mix. The turbid solution is kept standing for 2hrs. and mix before use.

ii. 50 ml glycerol+ 30ml HCl +300ml d/w + 100ml 95% ethyl alcohol or isopropyl alcohol + 75 g NaCl.

iii. Stock sulphate solution (100mg/l):- Dissolve 0.1479 g of Na_2SO_4 in 1000ml H_2O.

iv. Barium chloride crystal

v. Standard sulfate soln.: NIST std. 1000 mg/L SO_4

vi. Standard sulphate solution: - Prepare by diluting the stock solution in range between 1to 40 mg/l.

Interference: Colour or suspended matters in large amount interference in testing.

Procedure:

Filter the sample through 0.45µ, if there is any turbidity. Take 20 ml sample + 1ml HCl Soln. + 1ml conditioning reagent. Mix well and wait for 10 mins if glycerol reagent used and wait for 30 mins if gelatin reagent used and then take absorbance at 420 nm in spectrophotometer. The same is done with blank and standards.

Calculation:

From the calibration curve the concentration of sulphate directly determined.

No.16 Nitrate- nitrogen as NO_3 mg/L

Method name: - Chromo tropic acid method

Specification of the method: - ***IS-3025(part34)-1988, Reaff 2003.***

Equipment used: - Spectrophotometer and glassware

Ref. standard used: - NIST std. nitrate solution

Environmental condition:- i. Required for test: - Room temperature

Principle:

Two moles of nitrate nitrogen react with one mole of chromotropic acid to form a yellow reaction product having maximum absorbance at 410nm.

Reagents:

i. Stock nitrate soln.: Dissolve 0.7218 g of dry potassium nitrate in water and dilute to1000 ml. Preserve with 2 ml chloroform per liter. (1ml= 100 µg of nitrate nitrogen).

ii. Std. Nitrate soln. 10 ml stock nitrate solution is diluted to 100 ml1 ml = 10 mg NO_3 N

iii. Hydrochloric acid soln. 1 (N)

iv. Sulphite urea reagent. - Dissolve 5 g of urea+ 4g sodium sulphite + water to 1000ml.

v. Antimony reagent: - 500mg antimony + 80ml conc. H_2SO_4 cool + 20ml of ice water.

vi. Chromotropic acid reagent: - 100mg Chromotropic acid + 100ml conc. H_2SO_4. Store in a brown bottle.

vii. Con. H_2SO_4

Procedure:

i. Sample preparation: Take 2ml Sample + 1drop sulphite urea reagent place in a tray of cold water. + 2ml antimony reagent. Swirlan let stand for

4min.s in the bath + 1ml chromotropic acid wait 3min in cool water bath+ con H_2SO_4 up to10 ml.

ii. Standard preparation: Prepare standards 0.1-5.0 mg/l by diluting with d/w. 2 ml of each+ 1 drop sulphite urea reagent cool + 2 ml antimony reagent and wait 4 min + 1ml chromotropic acid wait 3min + con H_2SO_4up to 10 ml.

iii. Blank preparation: 2 ml d/w +1 drop sulphite urea reagent cool + + 2ml antimony reagent and wait 4 min + 1ml chromotropic acid wait 3min + con H_2SO_4 up to 10 ml. Stopper each flask and shake for 4 times .Wait 45 mints at room temperature and take absorbance at 410 nm.between15 min.24hrs. Concentration is obtained from absorbance curve.

Calculation:

Nitrate nitrogen, mg/l =µ g of nitrate in 10 ml final volume/volume in ml of sample taken for

No. 17 Nitrite-nitrogen as NO_2

Method name: - **NED method**

Specification of the method: - ***IS-3025(part-34)-1988, reaff 2003***

Equipment used: - Spectrophotometer and glassware

Ref. standard used: - Std. sodium-nitrite

Environmental condition:

i. Required for stabilization / storage: - Not applicable

ii. Required for test: - Room temperature

Principle:

Nitrite is determined by the formation of a redfish purple azo dye produced at pH 2 to 2.5 by coupling diazotized sulphanalic acid with NED dihydrochoride. The colour obeys beers law at wavelength 543nm.

Reagents:

i. Stock nitrite soln.: 1.232 gm $NaNO_2$ in 1000 ml distilled water. Preserved with 1ml chloroform.1 ml = 250 g NO_2 N

ii. Standardization of nitrite Soln. Pipette 50 ml std. 0.01 (M) $KMnO_4$, 5 ml conc. H_2SO_4 and 50 stock nitrite soln. shake gently and warm the soln. 70° to 80° C. Discharge permanganate colour by adding 10 ml portion of 0.025 M

$Na_2C_2O_4$. Titrate excess $Na_2C_2O_4$ with 0.01 M $KMnO_4$ to the faint pink endpoint. Carry a water blank through the entire procedure.

A = [(B X C) – (D X E)] X 7 / F

A = mg NO_2N / ml in stock $NaNO_2$ soln.

B = Total ml $KMnO_4$ used

C = (N) of $KMnO_4$

D = total ml $Na_2C_2O_4$ used

F = Stock $NaNO_2$ soln. used for titration

iii. Intermediate Nitrite soln. Calculate the vol. G of stock soln. from G = 12.5 /A, Dilute the vol. G to 250 ml distilled water. 1 ml = 50 g NO_2N

iv. Std. 0.01 (M) $KMnO_4$:
Dissolve 1.6 gm $KMnO_4$ in 1 L distilled water. Standardize the soln. by std. $Na_2C_2O_4$.

v. Sulphanilamide solution: - Dissolve 5gm of sulphanilamide in 50 ml con HCl + 300ml water. Dilute to500 ml with water.

vi. NED dihydrochloride: Dissolve 500gm NED dihydrochloride in 500 ml water. Store in dark bottle.

vii. 1:3 HCl.

viii. Sodium oxalate:-0.05 N. Dissolve 3.350 gm of sodium oxalate in 1000 ml water.

ix. Ferrous ammonium Sulphate 0.05N:-Dissolve 19.607 gm of ferrous ammonium sulphate in 20 ml con. H_2SO_4 and diluted to 1000 ml water. Standardized with standard dichromate.

Procedure:

Filtering sample 50ml + 1ml sulphanilamide solution wait 8min +1ml NED solution, mix and wait for 10 mins but not more than 2 hrs. And take absorbance at 543 nm. Do same with the standards and the blank and prepared the standards 2-25 mg/l.

Calculation: Mg/l

No. 18 Ammonia-nitrogen as NH_4 mg/L

Method: PHENATE SPECTROPHOTOMETRIC

Apparatus 1. Spectrophotometer used at 640nm with a cell of 1cm or longer

light path.

Reagents

Phenol solution:

Mixed 11.1 mL liquified phenol (>89%) with 95% V/V ethyl alcohol to a final volume of 100 mL. It is toxic, avoid personal exposure, discard after a week.

i. Sodium nitroprusside, 0.5%:

It is dissolved 0.5g sodium nitroprusside in 100 mL de-ionised water, store in amber bottle, discard after a month.

ii. Alkaline citrate:

Dissolved 200 g trisodium citrate and 10g sodium hydroxide in de-ionised water, dilute to 1L.

iii. Sodium hypochlorite solution, 5%: Commercial, replace every 2 months.

iv. Oxidizing solution:

Taken 100 mL alkaline citrate solution and mix with 25 mL sodium hypochlorite, prepare daily.

v. Stock ammonium solution:

Weighed 3.819g anhydrous, NH_4Cl, earlier dried at 100˚ C and cooled in desiccator, in ammonia free water and dilute to 1L; 1 mL = 1mg N = 1.22 mg NH_3.

vi. Standard ammonium solution:

Prepared dilutions from the stock ammonium solution, in a range appropriate for the concentration of the samples; prepare a calibration curve.

Procedure

a. Taken 25 mL sample in a 50 mL conical flask, and added with mixing, 1 mL phenol solution, 1 mL sodium nitroprusside solution, and 2.5 mL oxidising solution. Avoided light exposure by suitably covering the flasks at room temperature.

b. Prepared a blank and 2 other ammonia standards in the range, treating in the same way as sample, measure absorbance after 1h at 640 nm.

Calculation

Prepare calibration curve by plotting absorbance readings against ammonia concentration of standards, compute sample concentration from the standard curve.

No.19 Chloride as Cl mg/L

Method name: - **Argentometric method**

Specification of the method: - ***IS-3025(part32)-1988, Reaff 2003***

Equipment used: - Glassware

Ref. standard used: - NIST standard sodium chloride

Environmental condition:- i. Required for stabilization / storage: -Not applicable

ii. Required for test: - Room temperature

Principle:

Chloride is determined in a neutral or slightly alkaline solution by titrations with standard silver nitrate, using potassium chromate as an indicator. Silver chloride is quantitatively precipitated before red silver chromate is formed.

Reagents:

i. Potassium chromate indicator:

50 gm K_2CrO_4 dissolved in distilled water add $AgNO_3$ till definite red precipitate is formed. Allow to stand for 12 hours filter and dilute to 1000 ml.

ii. Standard Silver nitrate titrant 0.0141 (N):

2.395 gm of $AgNO_3$ is dissolved in 1 lit distilled water. Standardized against 0.0141N NaCl solution. 1ml=500 µg of Cl^- . Store in a brown bottle.

iii. Sodium chloride 0.0141 N:

` 824.0 mg NaCl is dissolved in 1 lit of distilled water.1 ml = 500 g Cl

iv. Aluminium hydroxide:

Dissolve 1.25g of aluminium potassium sulphate in distilled water upto 1ltr. Warmed to 60º. Let it stand for 1hr.

v. Phenolphthalein indicator solution

vi. Sodium hydroxide - 1 N

Sulphuric acid- 1N

viii. Hydrogen peroxide - 30%

Procedure:

Take 100 ml sample. If sample is highly coloured add 3ml of aluminium hydroxide suspension, mix , let settle and filter. If sulphide, sulphite or Thiosulphate is present, add 1ml of hydrogen peroxide and stir for 1min and titrate the sample in the pH range 7-10 adjusting with sulphuric acid or sodium hydroxide. Add 1ml of potassium chromate indicator solution and titrate with standard silver nitrate solution to a pinkish yellow end point. Standardized silver nitrate solution and establish reagent blank value by titration method.

Calculation:

Chloride mg/L = (A –B) X N X 35450/vol. of sample

where, A= ml $AgNO_3$ solution used for sample

B= ml $AgNO_3$ solution for blank

N= normality of $AgNO_3$ solution

No.20 Phosphate as PO_4 mg/L

Name of method: - Stannous Chloride method

Specification of the method: - **APHA 4500-P D**

Equipment used: - i. Spectrophotometer ii. Glassware
iii. Filtration assembly and filter paper

Principle:

Molybdophosphoric acid is formed and reduced by stannous chloride to intensely colored molybdenum blue. The minimum detectable limit in this method is 3µg P/L.

Reagent:

i. Phenolphthalein indicator aq. Solution

ii. Strong acid solution: Slowly add 300 ml conc. H_2SO_4 to about 600 ml distilled water. When cool add 4 ml conc. HNO_3 and dilute to 1 ltr.

iii. Ammonium molybdate reagent I : Dissolve 25 g ammonium molybdate $(NH_4)_6Mo_7O_{24}.\ 4H_2O$ in 175 ml distilled water. Cautiously add 280 ml conc. H_2SO_4 to 400 ml distilled water. Cool, add molybdate solution and dilute to 1 ltr.

iv. Stannous chloride reagent I: Dissolve 2.5g fresh $SnCl_2.2H_2O$ in 100 ml glycerol. Heat in a water bath and stir with a glass rod to hasten dissolution. This reagent i stable and requires neither preservatives nor special storage.

v. Standard phosphate solution: Dissolve in distilled water 219.5 mg anhydrous KH_2PO_4 and dilute to 1000 ml. 1ml=50 µ g PO_4 3-P.

Procedure:

Sample preparation:

To 100 ml sample containing not more than 200 µg P and free from colour and turbidity, add 0.05 ml (1 drop) phenolophthalein indicator. If sample turns pink add strong acid solution drop wise to discharge the colour. If more than 0.25 ml (5 drops) is required, take a smaller sample and dilute to 100 ml with distilled water after discharging the pink colour with acid.

a) Direct procedure:

i) Colour development:

Add with through mixing after each addition, 4 ml molybdate reagent I and 0.5 ml (10 drops) stannous chloride reagent I. Rate of colour development and intensity of colour depend on temperature of the final solution, each 1°C increase producing about 1% increase in colour. Hence hold samples, standards, and reagents within 2°C of one another and in the temperature range between 20 to 30°C. Prepare blank and standard as above. The range of standards for 10 cm path length is 0.007 -0.2 mg/l.

ii) Colour measurement:

After 10 min but before 12 min all the colour measurement should be done for the same specific interval at 690 nm. Measure the conc. for sample comparing the standards using blank.

b) Extraction procedure:

When increased sensitivity is desired or interferences must be overcome, phosphate is extract as follows: Pipette a 40 ml sample or one diluted to that volume into a 125 ml separating funnel. Add 50 ml benzene- isobutanol solvent and 15 ml molybdate reagent II. Close funnel and shake exactly for 15 s. If condensed phosphate is present ,any delay will increase its conversion to

orthophosphate. Remove stopper and withdraw 25 ml of separated organic layer, using a pipette with safety bulb. Transfer to a 50-ml volumetric flask, add 15 to 16 ml alcoholic H_2SO_4. Mix thoroughly. After 10min, but before 30min, read against the blank at 625 nm. Prepare blank by carrying 40 ml distilled water through the same procedure used for the sample. Read phosphate concentration from a calibration curve prepared by taking known phosphate standards through the same procedure used for samples.

c) Calculation:

i) Direct procedure:

mg P/L = mg P(in approximately 104.5 ml final vol)×1000 /ml sample

ii) Extraction procedure:

mg P/L = mg P(in 50ml final vol)×1000/ml sample

No.21 Sodium as Na mg/L

Name of the method: - Flame Photometry Method

Specification of the test: - ***IS-3025(part 45)-1993***

Equipment used: - Flame Photometer.

Reference standard used: - NIST standard sodium chloride

Environmental condition: - i. Required for stabilization/storage: - 4ºC (pH < 2 with HNO_3)

ii. Required for test: - Room temperature

Principle:

A flame photometer measures photoelectrically the intensity of colour imparted to the flame of a Meeker-type burner where the sample is introduced in to the flame under carefully standardized conditions. The intensity of colour is promotional to the sodium content in the sample. Sodium is determined at a wave length of 589 nm.

Reagents:

i. Stock Sodium Solution:

Dissolve in distilled water, 2.542 g of sodium chloride dried to constant mass at 1400C and make up to 1000ml with water. 1ml = 1mg of Sodium.

ii. Standard Lithium Solution:

Weigh rapidly 6.109 g of Lithium Chloride or 9.93 g of Lithium nitrate

dried overnight in an oven at 1050C Dissolve in water and make up to 100ml (1ml = 1 mg of Lithium.)

iii. Nitric Acid.

iv. Hydrochloric acid.

Procedure:

1. Pre-treatment of polluted and waste-water Samples:

Take 100 ml of Sample in a beaker and add 5 ml of Conc. Nitric acid and evaporate to dryness. Repeat this operation once again by adding conc. nitric acid. Dissolve the residue in minimum volume of conc. Hydrocholoric acid. Boil to dissolve, dilute to 50 ml with water filter and make up to 100 ml. Note: No preliminary treatment of sample is required except filtration if the sample contains only suspended material.

i. Direct –Intensity Measurement:

Prepare a blank and sodium standards in step amounts diluting the stock solution described in 10.1 for any of the following applicable ranges: 0 to 1.0 mg/L, 0-10 mg/L, or 0-100 mg/L, so that within each range there are equally spaced standards in tenths of the maximum. Starting with the height calibration standard and working towards the most dilute standard, measure emission at 589nm for Sodium. Repeat the operation with calibration and sample enough number of items to secure are liable average reading for solution. Construct a calibration curve, by plotting emission intensity verses concentration of each calibration standard on a linear graph paper. Determine sodium concentration of the sample solution from the respective calibration curve.

ii. Internal –Standard Measurement:

Add an appropriate volume of standard lithium solution to carefully measured volume of sample. Sodium standard and the blank, and then followed all the steps described in ii.

Calculation: - Sodium (mg/L) = Sodium in mg/L in portion x D

Where, D=dilution ratio = Sample in ml+ distilled water in ml

Sample in ml

No. 22 Potassium as K mg/L

Name of the method: - Flame Photometry Method

Specification of the test: - ***IS-3025 (Part 45)- 2008***

Equipment used: - Flame Photometer.

Ref. standard used: - NIST Standard Potassium solution

Environmental condition: -
i. Required for stabilization / Storage:-40c (pH< 2 with HNO_3)
ii. Required for Test: - Room temperature .

Principle:

A flame photometer measures photoelectrically the intensity of colour imparted to the flame of a Meeker-type burner where the sample is introduced in to the flame under carefully standardized conditions. The intensity of colour is promotional to the Potassium content in the sample. Potassium is determined at a wave length of 766.5 nm.

Reagents:-

i. Stock Potassium Solution:
Dissolve in distilled water, 1.907 g of Potassium chloride dried to constant mass at 1100C and make up to 1000ml with water. 1ml = 1mg of Potassium.

ii. Standard Lithium Solution:
Weigh rapidly 6.109 g of Lithium Chloride or 9.93 g of Lithium nitrate dried overnight in an oven at 1050C Dissolve in water and make up to 100ml. 1ml = 1mg of Lithium.

iii. Nitric Acid.

iv. Hydrochloric acid.

Procedure:

i. **Pre-treatment of polluted and waste-water Samples:**
Take 100 ml of Sample in a beaker and add 5 ml of Conc. Nitric acid and evaporate to dryness. Repeat this operation once again by adding conc. nitric acid. Dissolve the residue in minimum volume of conc. Hydrocholoric acid. Boil to dissolve, dilute to 50 ml with water, filter and make up to 100 ml. Note: No preliminary treatment of sample is required except filtration if the sample contains only suspended material.

ii. **Direct – Intensity Measurement:**
Prepare a blank and Potassium standards in step amounts diluting the stock

solution described in 10.1 for any of the following applicable ranges: 0 to 1.0 mg/L, 0-10 mg/L, or 0-100 mg/L, so that within each range there are equally spaced standards in tenths of the maximum. Starting with the height calibration standard and working towards the most dilute standard, measure emission at 766.5 nm for Potassium. Repeat the operation with calibration and sample enough number of items to secure are liable average reading for solution. Construct a calibration curve, by plotting emission intensity verses concentration of each calibration standard on a linear graph paper. Determine Potassium concentration of the sample solution from the respective calibration curve.

iii. **Internal –Standard Measurement**

Add an appropriate volume of standard lithium solution to carefully measured volume of sample. Potassium standard and the blank, and then followed all the steps described in ii

Calculation: Potassium (mg/L) = Sodium in mg/L in portion x D

Where, D=dilution ratio = Sample in ml + distilled water in ml/ Sample in ml

No. 23 Calcium as Ca mg/L

Method name: - EDTA Titrametric method

Specification of the method: - ***IS 3025 (part40):1991, Reaff: 2003***

Equipment used:- Hot plate, glassware

Ref. standard used: - $CaCO_3$ NIST std.

Environmental condition: -

i. Required for stabilization / storage: - 4ºC (pH < 2 with H_2SO_4)

ii. Required for test :- Room temperature

Principle:

In a solution containing both calcium and magnesium, calcium can be determined directly with EDTA when pH is (12-13) so that magnesium is largely precipitated as the hydroxide and an indicator is used which combines only with calcium.

Reagents

i. **Standard EDTA - 0.01 (M)**

3.723 gm of disodium salt of EDTA is dissolved in 1000 ml distilled water.

Standardize against standard calcium Solution. Cheek the strength of EDTA standard dizing against standard calcium solution.0.01M EDTA Solution =0.4008mg/l Calcium.

ii. **0.1 N HCl**

iii. **Stock calcium solution:**

Dry calcium carbonate (CaCO3) at 180˚C for 1 hr and cool in a desiccator. 2.50 gm $CaCO_3$ dried powder is suspended in 100ml water. Add slowly 0.1N HCl solutions to dissolve $CaCO_3$.Boil to expel dissolved carbon dioxide cool and transfer the solution to a 1000ml volumetric flask and diluted to mark with 0.1N HCL.

iv . **Standard calcium solution:** Dilute 100 ml of the stock calcium solution to 250 ml using 0.1N HCl. 1 ml=0.4008 mg Ca or 1 mg $CaCO_3$.

iv. **Sodium hydroxide: 1N**

Dissolve 40 gm NaOH in 1000 ml distilled water. The indicator solution is prepared by dissolving 150 mg of dye in100g of absolute ethylene glycol. It can also be prepared by mixing 200 mg of Murexide with 100 mg of solid sodium chloride and grinding the mixture to 355 to 300 microns which is unstable in alkaline medium.

Procedure:

Take 100ml sample in a 250ml conical flask +5ml conc. HNO_3 and covered with a wash glass. Evaporate it to a volume about 20ml by heating. Again add 5ml conc. HNO_3 and boil. Do it again and again until complete digestion but not make it dry before digestion. after digestion make it cool and diluted to 100ml. Select 50ml sample or sample diluted to 50 ml from above pre treatment sample so that calcium content is about 5-10mg. 50ml sample + 2 ml sodium hydroxide soln to get pH (12-13) + 0.1-0.2 g murex idée indicator and titrate against 0.01 (M) EDTA. Colour change from pink to purple.

Calculation:

Calcium (Ca) mg/l = A x B x 1000/V

Where A= ml of EDTA for titration

B= mass mg $CaCo_3$ equivalent to 1 ml EDTA solution

V= Volume of the sample

No.24 Magnesium as Mg mg/L

Method name: - Volumetric method using EDTA

Specification of the method: - ***IS 3025 (part46):1994***

Ref. Standard used: - Magnesium standard (NIST)

Environmental condition: - i. Required for stabilization / storage: - Not applicable

ii. Required for test: - Room temperature

Principle:

Water containing both calcium and magnesium is titrated with EDTA at pH 10, using Ferrochrome black-T as indicator, which estimates calcium in presence of magnesium. In a separate titration against EDTA at pH 12 to 13 using murex idée or Patton & Reeder´s indicator, calcium is selectively estimated. From these two values magnesium can be calculated.

Reagents:

i. **Buffer Solution:** 70g NH_4Cl+570ml 30% ammonia solution (relative density 0.88 to 0.90) in water and volume made up to 1ltr.

ii. **Standard EDTA- 0.01 (M):** 3.723 gm of disodium salt of EDTA is dissolved in 1000 ml distilled water and standardized with standard calcium solution.

iv. **Erichrome black T indicator**: - Dissolve 0.5 g of EBT+4.5g of hydroxylamine in 100 ml rectified spirit (ethanol or methanol).

iv. Potassium Cyanide Solution-10 %(m/v)

Hydroxylamine Hydrochloride Solution – 10% (m/v)

v. Tri ethanolamine Solution-10 %(m/v)

Procedure:

i. Determination of calcium as per Test No 14

ii. Determination of magnesium: Take a suitable aliquot of the solution, expected to contain approximately 10 to 30 mg of calcium and magnesium in 500 ml conical flask. Add 10 ml hydroxylamine hydrochloride solution, 2ml potassium cyanide solution and 25ml of tri ethanolamine solution. Dilute to 150 to 200 ml and sufficient quantity of buffer solution to bring the Ph to 10.0 + 0.1. Add 3 to 4 drops of EBT indicator solution and titrate with 0.01 M EDTA solution till the red colour changes to pure blue end point free from violet tinge.

Calculation:

Magnesium Hardness (mg/l) = 0.02435x1000x (V_2-V_1)/V

Where V=Volume of Sample

V_1= Volume of EDTA for calcium

V_2=Volume of EDTA using EBT indicator.

No.25. Iron as Fe mg/L

Method name: 1, 10 Phenanthroline method

Specification of the method: ***IS-3025(part 53):2003***

Equipment used: Spectrophotometer 510 nm

Ref. standard used: NIST std. Fe

Environmental condition: i. Required for stabilization / storage: - 4ºC (pH < 2 with HNO_3)

ii. Required for test :- Room temperature

Principle:

Iron in the soln. is reduced to the ferrous state by boiling with HCl & hydroxylamine hydrochloride & treated with 1, 10 phenanthroline at PH 3.3.

Reagent:

i. Stock iron soln.: In a 250 ml beaker 20 ml conc. H_2SO_4+50 ml d/w+1.404g ferrous ammonium sulphate+0.1N potassium permanganate drop wise until a faint pink colour persists and then volume make up to 1000 ml. 1 ml=200µg Fe.

ii. 11 N HCl

iii. Hydroxylamine soln: Dissolve 10g hydroxylamine hydrochloride in 100ml water. Stable for one week.

iv. Ammonium acetate buffer soln: Dissolve 250g in 150ml water. Add 700ml glacial acetic acid.

v. 1, 10-phenanthroline soln: Dissolve 0.1g in 100ml water & add 2 drops conc. HCl. Stable for one week.

vi. Di isopropyl ether

Procedure:

50 ml sample+ 2ml HCl+ 1ml Hydroxylamine soln + D/W up to 50 ml. Boil to reduce the volume. to 20 ml. Cool & add 10ml 1, 10 phenanthroline + 10 ml ammonium acetate buffer soln. & diluted to 100ml with d/w. Do the same with the blank and the standards. Wait for 15 min & take absorbance at 510 nm. Conc. of Fe

obtained from conc.-abs curve. Prepare the standards in the range 10-100 µ g iron.

Calculation:

Mg / l Fe= µg of Fe from curve/vol. of sample

26. Lead as Pb mg/L

Method name: - Di thizone method

Specification of the method: - ***IS-3025(part 47):1994***

Equipment used: - Spectrophotometer, pH meter

Ref. standard used: - NIST std. Pb

Environmental condition:- i. Required for stabilization / storage: - 4ºC (pH < 2 with HNO_3)

ii. Required for test: - Room temperature

Principle:

Acidify sample containing lead is extracted with dithizone soln in chloroform. The extraction is carried out in presence of strong ammonical citrate cyanide agent at pH 10 to 11.5: The absorbance measure in spectrophotometer at 510nm of the chloroform extract.

Reagent:

i. Stock lead soln.: 0.1599 g lead nitrate (99.5% pure) +200 ml d/w+10 ml conc. HNO3+d/ up to 1000 ml. 1 ml=100 µg Pb

ii. Conc. HNO_3 (18N)

iii. 20% HNO_3 (v/v)

iv. 1% & 10% NH_4OH (v/v)

v. Citrate cyanide reducing soln: Dissolved 200g ammonium citrate & 10 g sodium sulphite + 5g hydroxylamine hydrochloride + 20g KCN in water & diluted to 500ml & mix with 1lt of conc. NH_4OH. Stock dithizone solution: - Dissolve 25mg dithizone in 50ml chloroform. Filter and then

vi. Wash with chloroform and transfer the filtrate into a 500 ml separating funnel. Add 100ml of 1% ammonium hydroxide and shake for one min, transfer chloroform layer to 250ml saparatory funnel retaining orange red aqua's layer 500ml separatory funnel. Add 2ml 1:1 HCl mix till dithizone precipitated and orange red colour appears with three 25ml portion of chloroform. Dilute the combine extract to 250 ml with chloroform. (1ml = 100µg dithizone)

Procedure:

i. Sample preparation and procedure: Digest all sample for dissolve and total as per as digestion procedure using HNO_3-H_2SO_4, & HNO_3-$HClO_4$.

ii. Take 100ml of the acidify sample (pH-2) + 20ml 20 %(v/v) HNO_3 filter if required.

iii. Transfer it to a 250 ml separating funnel. Add 60ml ammonical citrate-cyanide mix and cool at room temperature + 10ml Dithizone working solution. Shake the funnel about 30 sec. Discard the chloroform layer and take the absorbance at 510nm. Do the same with the blank and the standards.

Calculation: mg /L Pb= µg Pb obtained from curve/vol. of the sample in ml

No. 27 Copper as Cu mg/L

Method name: Neucruprine method

Specification of the method: ***IS-3025(part 42)***

Equipment used: Spectrophotometer at 457nm

Ref. standard used: NIST std. Cu

Environmental condition:

i) Required for stabilization / storage :- 4ºC (pH < 2 with HNO_3)

ii) Required for test :- Room temperature

Principle:

Copper (II) is reduced to copper (I) by hydroxylamine hydrochloride and the pH of the solution is adjusted to 5 by sodium citrate solution. Copper (I) forms a soluble yellow complex with neucruprine.

Reagents:

i. Stock. copper soln.: 0.2g of copper metal+6ml of 1:1 HNO_3 warm+1ml H_2SO_4 evaporate to dryness and diluted to 1 ltr. with d/w. 1 ml=200µg Cupper II.

ii. Ammonium hydroxide

iii. Chloroform

iv. HCl

v. Hydroxylamine hydrochloride Solution: 40g of hydroxylamine hydro chloride+200 ml d/w.

vi. Isopropyl alcohol, vii. HNO_3, viii. H_2SO_4

Neucruprine solution: - 0.1 g Neucruprine in 50 ml isopropyl alcohol and diluted to 100ml with d/w.Sodium citrate solution: - 250g sodium citrate is dissolve in distilled water and volume upto 1000ml. Add 10ml neucruprine + 10ml hydroxylamine hydrochloride solution and extract it with chloroform discarding
chloroform layer.

Procedure:

i. Sample preparation: If interfering substance are present than take100ml sample + 1ml H_2SO_4+ 5ml HNO_3 heating to dryness. Repeated the treatment with 5ml HNO_3 + 5ml H_2O_2 again evaporate to dryness. Dissolve the sample with 80ml of water boil and cool and filtered Adjust the pH to 4 to 6 by adding ammonium hydroxide +0.2ml HCl and diluted the volume to 100 ml with d/w.

ii. Transfer 50ml sample to a 250ml separating funnel + 5ml hydroxylamine hydrochloride solution + 10ml neucruprine solution shack well. + 20ml chloroform and shack for one min. Separate the chloroform layer and collect it in a dry flask. Extract the same once again combine the extract and dilute to 50 ml with isopropyl alcohol

iii. Prepared the blank and the standards with the same procedure and take the absorbance at 457nm.

iv. Prepare standards 0.05,0.1,0.5.1.0,5.0mg/l upto 50ml.

Calculation:

$$mg / l\ Cu = M \times 1000/V$$

where, M= mass in mg of sample from curve

V= vol. of sample in ml

No. 28. Zinc as Zn mg/L

Method name: - Zincon method

Specification of the method: - ***IS 3025 (part 49): 1994, Reaff 2003***

Equipment used: - Spectrophotometer

Ref. standard used: - NIST std. Zn

Environmental condition: i. Required for stabilization/storage: - 4ºC (pH <with HNO3) ii. Required for test: - Room temperature

Principle:

Zinc (II) forms a soluble blue complex with zincon at pH 9.0. The colour complex is suitable for Spectrophotometric measurement at 620 nm.

Reagent:

i. Std. zinc soln. 1000 mg/L

ii. NaOH soln- 40 g/L

iii. NaOH soln- 240 g/L

iv. KCN soln: 1g in water diluted to 100ml.

v. Chlorohexanone soln: 10g in 100ml water

vi. Zincon soln: Dissolve 0.325g of zincon reagent in 100ml methanol by heating. Cool and diluted to 250 ml with methanol. Store in a brown bottle.

vii. Borate buffer soln.: Dilute 213 ml of 1M NaOH soln to 500ml with water & dissolve 37.3g of KCl and 31 g boric acid and volm. Make upto 1 Lt. with water.

viii. Conc. HCl

ix. Zinc (II) soln.:

Dissolve 0.274g of zinc sulphate in 200ml of water & diluted to 1Lt. 1ml = 0.1 mg of Zn.0.2

Procedure:

50 ml sample+1ml HCl boil for 5 min. Cool & adjust the pH to 7 with NaOH soln. Make up the vol. up to 50 ml with d/w. Take 10 ml of the soln. + 0.5g sodium ascorbate+1ml cyanide soln+5ml buffer soln+3ml Zincon soln + 1ml chlorohexanone soln. and vol. make upto 500ml. Prepare blank and standards same as above. Take the absorbance at 620 nm. Determine the conc. of Zinc from the curve. Prepare the standards in the range 0.02-5.0mg/l.

Calculation:

Zinc, mg/L = $M \times 1000/V$

where, M= mass of Zn present in mg in the sample

V= vol. of sample

D.iv. Cu, Zn, Pb ESTIMATION FROM MACROPHYTE BIOMASS FOR PHYTOREMEDIATION STATUS STUDY

29. Estimation of Cu, Zn, Pb from Macrophyte biomass (Whole Plant body separatly) of ***Lemna perpusilla*** **Torry,** ***Azolla pinnata*** **R.Br,**

Salvinia cucullata. **Analysis done by the Method of Graphite Furnace Atomic Absorption Spectrometry *(GFAAS, Model-Analytik Jena Vario-6)***

Principle:

Graphite furnace atomic absorption spectrometry (GFAAS) (also known as Electro thermal Atomic Absorption spectrometry (ETAAS)) is a type of spectrometry that uses a graphite-coated furnace to vaporize the sample. Instead of employing the high temperature of a flame to bring about the production of atoms from the sample and it is a non-flame method involving electrically heated graphite tubes or rods.

Graphite tube samples are placed directly in the graphite furnace which is then electrically heated. Beam of light passes through the tube three stages:

i. Drying of sample
ii. Ashing of organic matter (to burn off organic species that would interfere with the elemental analysis.
iii. Vaporization of analyte atoms.

The sample is vaporized in the heated graphite tube and the amount of light energy absorbed in the vapour is proportional to atomic concentrations. The free atoms will absorb light at frequencies or wavelengths characteristic of the element of interest. Within certain limits, the amount of light absorbed can be linearly correlated to the concentration of analyte present.

Digestion process:

Nitric acid -- Hydrochloric acid digestion for Cu, Zn and Pb

i. Taken suitable volume of sample in a beaker.(1 gm dry mass of plants)
ii. Added 10 ml of nitric acid and added 10 ml 1+1 HCl
iii. Place beaker on a hot plate until volume has been reduced to near 25 ml
iv. Sample did not dry during digestion. Care was taken that time.
v. Filter sample by whatman no. 42.
vi. Make suitable final volume.

Calculation:- Metal concentration (mg/l) = **A × B/ C**

Where, A = Concentration of metal in digested solution, mg/l
B = sample taken for digestion, ml
C = final volume of digested solution, ml