PRACTICAL MANUAL
OF
ENVIRONMENTAL SCIENCE

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Practical Manual

Course: **Environmental Science**
***Course No*. BIRM 301**
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CERTIFICATE

Certified that this is a bonafide record of practical work done by Ms/Mr_____ with I.D.No._____for the course Environmental Science, course No.: BIRM 301 during the *First/Second* Semester of 20.. to 20.. .

Signature of Course-in-charge

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Expt. No.: 1 **Date:**

COLLECTION, PROCESSING AND STORAGE OF EFFLUENT SAMPLES

Introduction

Water pollution is mainly caused by two sources such as point and non-point. This depends on how the pollution enters the water. Point sources are sources of pollution situated at one location, often a specific outlet (discharge) pipe. Example is effluent from factories and wastewater treatment plants. The meaning of the term 'effluent' is "flowing forth out of". As per the Clean water Act of 1977, effluent is a discharge from a point source, and the legislation specifies allowable quantities of pollutants. The discharges are regulated under section 402 of the act, and the standards must be met before they are released into surface waters. Water sampling is an important factor for evaluation of its quality and it includes two aspects namely (a) when and where to sample (b) how to transfer the samples with minimal changes to the place of analysis.

Aim

To collect a portion of material small enough in volume to be transported conveniently and handled in the laboratory still accurately representing the material being sampled.

Materials required

Samplers, sample containers, cleaning agents, preservatives, tags, labels.

Samplers

Surface samples are collected in a jug/bucket/bottle. For collecting subsurface samples water samplers are commonly used. Different water samplers namely Ruttner water sampler, Von Dorn water sampler, Thermos-flask water sampler, pump samplers *etc.* are available.

Sample containers

There are two basic choices in containers *viz*., glass or plastic. In both the cases a tightly fitting screw cap top is essential. Adsorption or ion exchange on the glass surface invalidates the use of glass bottles for storage of water in which heavy metals are to be estimated. As compared to glass bottles, plastic bottles are inexpensive and less fragile with less ion exchange problem. Among the plastics, high density linear polythene may be used. Containers made of fluorinated polymers such as polytetrafluoroethylene (PTFE) can be preferred for sampling organics.

Cleaning

Plastic bottles including Teflon may contain impurities which will result in sample contamination. The method of cleaning depends on the use to which the bottles will be put. In general bottles should be cleaned with detergent, rinsed thoroughly with tap water and finally with distilled or de-ionized water. A non ionic detergent used in hot water is the best when heavy metals are to be analyzed. Presoaking of these in 1:1 HNO_3 or 1:1 HCl is always advisable.

Collection of samples

As there are random variations in both analytical procedures and constituents of the effluent, a single sample may be insufficient to get reliable data. The number and distance between sampling stations depend on the physical size of the study area, variability or gradients in the processes, which control the distribution of the investigated parameter. For most physical and chemical analyses, a 2 litre sample is sufficient.

Types of Samples

Grab or catch sample: The sample collected at a particular time and place which represent only the composition at that time and place. When a source is known to be constant in composition over a considerable period of time, then such a source may be represented well by single grab samples.

When a source is known to vary with time, grab samples collected at suitable intervals and analyzed separately can document the extent, frequency, and duration of these variations. Choose sampling intervals on the basis of the expected frequency of changes, which may vary from as little as 5 min to as long as 1 h or more. Seasonal variations in natural systems may necessitate sampling over months. When the source composition varies in space *i.e.,* from location to location rather than time, collect samples from appropriate locations that will meet the objectives of the study (for example, upstream and downstream from a point source etc). Take every possible precaution to obtain a representative sample.

Composite sample: It refers to the mixture of grab samples collected at the same sampling point at different times. A composite sample representing a 24 hours period is considered standard for most determinations. For determining components or characteristics subject to significant unavoidable changes on storage, composite samples should not be used.

Integrated sample: For certain purposes, the information needed is provided best by analyzing the mixtures of grab samples collected from different points simultaneously or as nearly so as possible. Such mixtures sometimes are called integrated samples.

Sampling Methods

Manual sampling: Manual sampling involves minimal equipment but may be unduly costly and time-consuming for routine or large-scale sampling programs. It requires trained field technicians and is often necessary for regulatory and research investigations for which critical appraisal of field conditions and complex sample collection techniques are essential. It is required in case of waters containing oil and grease.

Automatic sampling: Automatic samplers can eliminate human errors in manual sampling, can reduce labor costs, may provide the means for more frequent sampling, and are used increasingly. Be sure that the automatic sampler does not contaminate the sample. For example, plastic components may be incompatible with certain organic compounds that are soluble in the plastic parts or that can be contaminated (e.g., from phthalate esters) by contact with them. If sample constituents are generally known, contact the manufacturer of an automatic sampler regarding potential

incompatibility of plastic components. Program an automatic sampler in accordance with sampling needs. Carefully match pump speeds and tubing sizes to the type of sample to be taken.

Sorbent sampling: Use of solid sorbents, particularly membrane-type disks, is becoming more frequent. These methods offer advantages of rapid, inexpensive sampling if the analytes of interest can be adsorbed and desorbed efficiently and the water matrix is free of particulates that plug the sorbent.

Preservation Chemical preservatives should be used only when they are shown not to interfere with the analysis being made (Table 1). For analysis of cations such as Al, Cd, Cr, Cu, Fe, Pb, Mn, Ag and Zn the sample should be collected in a separate clean bottle and acidified with HNO_3 to a pH below 2.0 to minimize precipitation and adsorption of these to container walls. Mercuric chloride @50 mg per litre of sample can be used as preservative to check biological activity. Addition of 2 or 3 drops of toluene is also an option to prevent bacterial activity.

Field log Book

This should include purpose of sampling, location of sampling point, name and address of the field contact, producer of material being sampled and address. Suspected sample composition including concentrations, number and volume of samples taken, description of sampling point and sampling method, date and time of collection, collector's identification number and sample distribution with transport particulars. Protect the log book and keep it in safe place.

Chain-of-custody record

Properly designed and executed chain-of-custody forms will ensure sample integrity from collection to data reporting. The process of tracing the possession and handling of the sample from the time of collection through analysis and final disposition is referred to as "chain-of-custody" and is required to demonstrate control of samples particularly, when the data are to be used for regulation or litigation or logical interpretations. This should accompany each sample or groups of samples. The record includes sample number, signature of the collector, date, time, and address of collection, sample type, signatures or persons involved in the chain of possession and inclusive of the dates of possession.

Storage of Samples

After examining the information accompanying the sample and verification regarding any tampering collection, the sample should be stored in a secured place. For most of the physical and chemical analysis some holding time for sample is permissible. Parameters which need immediate estimation include parameters like pH, free CO_2, alkalinity, dissolved oxygen and sulphide. Most of the samples may be stored at 4^oC.

Sample seals and labels

Gummed paper labels or tags are generally adequate. Labels should include name of the collector, date, hour, exact location, water temperature, water level, post sampling handling and steam flow. Labels should be filled in before or at the time of sampling with water proof ink.

Sample Delivery to the Laboratory

This should be done as soon as practicable to the sample custodian.

Precautions in collection and preservation of samples

- It is always desirable to rinse the water sampler or sampling container thoroughly with the water to be collected by it.
- Hot water samples collected under pressure should be cooled while they are still under pressure.
- Well water should be pumped sufficiently to ensure that sample collected represents ground water source.
- Surface scum should be avoided while sampling.
- Excessive turbulence causes potential loss of volatile constituents and toxic vapours, hence areas of excessive turbulence are to be avoided.
- In case of organic determinations sample should be filled full in the bottle. Samples if shipped an air space of about one percent is to be left.
- If preservatives are used, add them to the sample bottle initially so that all portions of the composite are preserved as soon as collected.
- Dissolved oxygen if to be estimated by Winkler's method should immediately be fixed by adding manganous sulphate and alkaline potassium iodide solutions.

Table Summary of sampling and handling requirements

S No.	Test to be conducted	container[+]	Sample type!	Minimum sample size - ml	Preservation procedure	Maximum storage time
1	Acidity	P,G(B)	g	100	Refrigerated*	24 hours
2	Alkalinity	P,G	g	200	Refrigerated	24 hours
3	BOD	P,G	g, c	1000	Refrigerated	6 / 48 hours
4	COD	P,G	g, c	100	Analyze as soon as possible or add H_2SO_4 to bring pH to < 2	7 days / 28 days
5	Chlorine residue	P,G	g	500	Refrigerated and to be analyzed immediately	0.5 hours
6	Chlorophyll	P,G	g	500	30 days in dark	30 days
7	Hardness	P,G	g	100	Add HNO_3 to bring pH to < 2	6 months
8	Metals	P(A),G(A)	g, c	--	For dissolved metals filter immediately and HNO_3 to bring pH to < 2	6 months
9	pH	P,G	g	--	Analyze immediately	0.5 hours
10	Turbidity	P,G	g, c	--	Analyze same day	24 hours
11	Solids	P,G	g, c	--	Refrigerated	7 days
12	Dissolved oxygen	G, BOD bottle	g	300	Analyze immediately	0.5 hours
13	Oil and grease	G,wide-mouth calibrated	g	1000	Add HCl or H_2SO_4 to pH <2, refrigerate	28 days

* Refrigerated means sample is stored at 4^oC in dark

[+] P = Plastic, G = Glass, P (A), G (A) = rinsed with 1+1 HNO_3, G (B) =glass, borosilicate

! g—grab; c---composite

Expt. No.: 2 **Date:**

Determination of Chemical Oxygen Demand in Waste Water Sample

Introduction

Chemical oxygen demand (COD) is a measure of the ability of chemical reactions to oxidize matter in an aqueous system. The major advantage of the COD test is the short time required to carry out rather than the longer 5 days procedure for BOD. The limitation of the COD test lies in its inability to differentiate between the biologically oxidizable and biologically inert material. For a raw, domestic waste water, the COD/BOD_5 ratio is 1.5-3.0/1.0 approx. Higher ratios indicate toxic, non biodegradable contaminants. Though COD is a precise test, this is being done on few samples as the spent solutions generated are hazardous as they are acidic and contain mercury, chromium and silver.

Aim

To estimate COD of the waste water sample

Apparatus

COD reflex unit consisting flat bottom flask with ground glass mouth (250 ml) with leibig (straight tube, single surface) condenser (30 cm), hot water bath or heating mantle, burette and pipette

Reagents

1. Standard potassium dichromate ($K_2Cr_2O_7$) 0.25N: Dissolve 12.25 g of $K_2Cr_2O_7$ in distilled water and make up the volume to 1000 ml with distilled water in a volumetric flask .
2. $HgSO_4$ and $AgSO_4$ crystals
3. Concentrated H_2SO_4
4. Ferroin indicator: Dissolve 0.695 g of ferrous sulphate ($FesO_4,7H_2O$) and 1.485 g of 1:10 phenanthroline in distilled water and make up the volume to 100 ml.
5. Standard ferrous ammonium sulphate [Fe $(NH_4)_2$ $(SO_4)_{2}$}]. 0.1N solution (0.1N=0.1M): Dissolve 39.2 g of ferrous ammonium sulphate in distilled water, add 20 ml of conc. H_2SO_4 and make up the volume to 1000 ml with distilled water.

Principle

The organic matter present in the given sample of wastewater is oxidized to CO_2 and H_2O by refluxion with a known excess amount of chromic acid ($K_2Cr_2O_7 + H_2SO_4$). The left over $K_2Cr_2O_7$ is titrated against standard ferrous ammonium sulphate (FAS) by using ferroin as indicator till bluish green colour changes to reddish brown. Simultaneously blank is conducted to know the actual amount of $K_2Cr_2O_7$ consumed for oxidation which is proportional to the oxygen required to oxidize the organic matter.

Procedure

- Place a pinch of mercuric sulphate in the flask of reflux unit and take 20 ml of the sample using pipette into the flask.

- Add 10 ml of 0.25N $K_2Cr_2O_7$ through pipette.
- Add a pinch of silver sulphate followed by 30 ml of concentrated H_2SO_4.
- Attach liebig condenser to the mouth of flask and heat the flask on a hot water bath or heating mantle for at least 2 hours to reflux the contents.
- Cool the flask, detach from unit and dilute the contents to about 150 ml by adding distilled water.
- Add 2-3 drops of ferroin indicator and titrate the contents of the flask with 0.1N ferrous ammonium sulphate solution till bluish green colour changes to reddish brown.
- Run simultaneously a blank without sample, with distilled water in a similar manner.

Precautions

- For complete oxidation of organic matters, it is necessary to see that quantity of H_2SO_4 should be equal to that of 'sample plus dichromate'.
- For accuracy, the sequence of making mixture should be $HgSO_4$, sample (Swirl), $K_2Cr_2O_7$ and conc. H_2SO_4 (slowly with swirling).
- After refluxing, cool the contents, wash the condenser and then titrate.

Interference ions removal

- The interference caused by chlorides can be eliminated by the addition of mercuric sulphate (Hg SO_4) to the sample prior to the addition of other reagents.
- Addition of silver sulphate to concentrated H_2SO_4 promotes the oxidation of straight chain aliphatic and aromatic compounds like acetic acid, amino acids etc as a catalyst.
- Nitrates exert a COD of 1.1 mg/mg N and this interference can be eliminated by the addition of 120 mg of sulphamic acid while preparing the standard potassium dichromate. When 20 ml sample and 10 ml dichromate is taken, this can take care of NO_2-N concentrations up to 6 mg/l.

Observations:

S. No	Volume of taken	Ferrous ammonium sulphate rundown (ml)		Titre value (Final –Initial) (ml)
		Burette reading (Initial)	Burette reading (Final)	
Sample				
1	10 ml			
2	10 ml			
3				
Blank				
1	10 ml			
2	10 ml			
3				

Calculations

1000 ml of 1N FAS = 8 gm of O_2

1000 ml of 0.1N FAS = $\frac{0.1}{1}$ x 8 gm of O_2

Titre value with sample = A ml

Titre value without sample (blank) = B ml

Amount of 0.1N FAS actually utilized for oxidation = (B – A) ml = T ml

T ml of 0.1N FAS = $\frac{T}{1000}$ x 0.1 x 8 gm of O_2

Volume of sample taken (V) = 20 ml

∴ 20 ml of sample = $\frac{T}{1000}$ x 0.1 x 8 gm of O_2

Then 1000 ml of sample = $\frac{1000}{20}$ x $\frac{T}{1000}$ x 0.1 x 8 gm of O_2

$= \frac{T}{20}$ x 0.1x 8gm of O_2

∴COD as mg of O_2 L^{-1} = $\frac{T}{20}$ x 0.1 x 8 x1000

Results and Inference:

Expt. No.: 3 **Date:**

Determination of Dissolved Oxygen in Waste Water sample

Introduction

Dissolved oxygen (DO) refers to the amount of oxygen dissolved in water and is particularly important for aquatic life. The amount of dissolved oxygen often determines the number and types of organisms living in that body of water. The amount of oxygen water can hold depends up on temperature, pressure and salinity. Many lakes and ponds have anoxic (oxygen deficient) bottom layers in the summer because of decomposition processes depleting the oxygen. Adequate dissolved oxygen is necessary for good quality of water. As dissolved oxygen in water drop below 5.0 mg/l, aquatic life is put under stress. Oxygen levels that remain below 1-2 mg/l for few hours can result in large fish kills.

Aim

To determine the amount of dissolved oxygen present in the given waste water sample by Winkler's method.

Apparatus

BOD bottles (100-300 ml), conical flask, burette and pipette

Reagents

1. Manganous sulphate solution: dissolve 96 g of $MnSO_4.4H_2O$ or 80g of $MnSO_4.2H_2O$ in 200 ml of previously boiled distilled water and filter the same if solution is not clear.
2. Alkaline potassium iodide solution: Dissolve separately 75 g of potassium iodide and 350g of potassium hydroxidein distilled water, mix the two solutions and make th. Dissolve the chemicals in 200 volume to 500ml.
3. Sodium thiosulphate solution (0.025N): Dissolve 6.205g of sodium thiosulphate in previously boiled and cooled distilled water and make up the volume to 1 litre. To this add a pellet of sodium hydroxide as a preservative and preserve the solution in coloured bottle. [For accurate results Standardization of sodium thiosulphate can be done using standard potassium dichromate. End point is from blue to colourless if starch as used as indicator]
4. Starch indicator: dissolve 1 g of starch in 100 ml of warm distilled water and add few drops of toluene or formaldehyde as preservative.
5. Concentrated sulphuric acid

Principle:

The "Winkler's" method is based on the fact that, when manganous sulphate is added to the sample containing alkaline potassium iodide, manganous hydroxide is formed. Dissolved Oxygen of the sample combines with $Mn(OH)_2$ and forms higher hydroxides namely basic manganic oxide. In the presence of iodine and on subsequent acidification the basic manganic oxide revert to the divalent state and liberate iodine equivalent to that of dissolved oxygen originally present in the sample. The liberated iodine is titrated with standard solution of sodium thiosulphate using starch as an indicator.

Chemical reactions

$MnSO_4 + 2KOH \rightarrow Mn(OH)_2 + K_2SO_4$ (white ppt)

$2\,Mn(OH)_2 + O_2 \longrightarrow 2MnO(OH)_2$ (Basic manganic oxide, brown coloured)

(Dissolved oxygen)

$MnO(OH)_2 + 2H_2SO_4 \rightarrow Mn(SO_4)_2 + 3\,H_2O$

(manganic sulphate)

$Mn(SO_4)_2 + 2KI \rightarrow MnSO_4 + K_2SO_4 + I_2$

$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$

Procedure

- Pipette out 50 ml of sample into a glass stoppered BOD bottle.
- Add 1 ml each of manganous sulphate and alkali- iodide-azide solution using separate pipettes (if the solution volume exceeds 200 ml, add 2 ml each of the reagent)
- Close the bottle without any entrapped air and shake well by inverting the bottle at least for about 1 minute.
- Brown precipitate formed is allowed to settle down. Sample at this stage can be stored for few days if required
- Add 2 ml of conc. H_2SO_4 and shake well to dissolve the precipitate formed.
- Transfer the whole content or known part into a conical flask and titrate the contents with standard sodium thiosulphate solution till the contents turns to straw yellow.
- Add 2 ml of starch solution. Now the contents change to blue colour.
- Continue the titration till the disappreance of blue colour, that is to colourless. Note down the titre value.

Interference:

Addition of sodium azide in alkaline potassium iodide solution avoids the interference due to organic matter and chlorides present in sample. For this purpose to prepare alkali-iodide-azide solution, dissolve 5 g sodium azide (NaN_3) in 20 ml distilled water in a beaker and then pour this azide solution into the alkali-iodide solution and mix well.

Precautions:

- Samples have to be collected in BOD bottles. Fill the bottles without entrapment of any air.
- DO value may change from point of collection to the analysis because of changes in temperature and also occurrence of biological reactions with time. In order to obtain correct DO value, the sample must be "fixed" immediately after collection. Fixing is done by adding 2 ml of manganous sulphate solution and 3ml of alkali-iodide-azide to the sample in BOD bottle by keeping the tip of the pipette below the surface of the liquid. Preserve the fixed sample by keeping at 4^0C in dark.

Observations:

S. No	Volume of Sample taken (ml)	Sodium thio sulphate rundown(ml)		titre value (ml) (Final – Initial)
		Burette reading Initial	Burette reading Final	
1	50 ml			
2	50 ml			

Calculations

1000 ml of 1N $Na_2S_2O_3$ = 8 gm of O_2

1000 ml of 0.025N $Na_2S_2O_3 = \frac{0.025 \times 8}{1}$ gm of O_2

Titre value = x ml

$\therefore$x ml of 0.025N $Na_2S_2O_3 = \frac{x}{1000} \times \frac{0.025}{1} \times 8$ gm of O_2

Volume of sample taken (V) = 50 ml

$\therefore$ 50 ml of sample = $\frac{x}{1000} \times \frac{0.025}{1} \times 8$ gm of O_2

Then 1000 ml of sample = $\frac{1000}{50} \times \frac{x}{1000} \times \frac{0.025}{1} \times 8$ gm of O_2

$= \frac{x}{50} \times 0.025 \times 8$ gm of O_2

$\therefore$DO in (mgL^{-1}) = $\frac{x}{50} \times 0.025 \times 8 \times 1000$

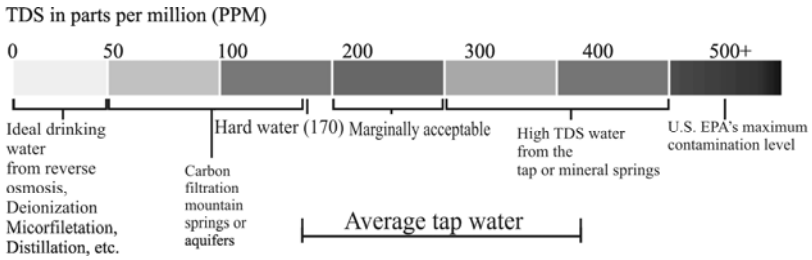
Results and Inference:

Expt. No.: 4 **Date:**

Determination of Total Dissolved Solids in Waste Water Sample

Introduction

Dissolved solids are minerals in solutions, typically measured in parts per million (ppm). This may also include organic substances as in the case of polluted waters. However, determination of the dissolved solids does not give a clear picture of kind of pollution. Water that contains excessive amounts of dissolved solids is unfit for drinking. Drinking water standards typically allow a maximum of 250ppm, the threshold for tasting sodium chloride; by comparison, in ocean water it ranges from 33,000 to37,000 ppm. High concentrations of dissolved solids, abut 3000 mg L^{-1} produce distress in livestock and not fit for use in industry. Use of water with high amounts of dissolved solids lead to scaling of boilers, corrosion resulting in degraded quality of the product.Dissolved solids buffer acid precipitation; lakes with low levels are especially vulnerable. High levels occur in runoff from newly- disturbed landscape, such as strip mines and road construction.



Aim

To find out the amount of total dissolved solids in the given waste water sample gravimetrically

Apparatus

Porcelain dish, desiccator, water bath, filter paper and funnel

Principle

A known volume of water is evaporated to dryness and the quaintly of soluble salts present is estimated gravimetrically.

Procedure

- Filter 50 ml of sample through whatman N0.4 filter paper in a pre-weighed porcelain dish.
- Evaporate the sample on hot water bath until whole water is evaporated

- Note the weight of porcelain dish after cooling it in a desiccator and calculate the total dissolve solids (TDS).

Observations

Initial weight of dish = W_1 gm

Volume of sample taken = 50 ml

Weight of porcelain dish after evaporation of sample = W_2 gm

Calculations

Weight of total dissolved solids = (Weight of dish along with dry solid) - (Initial weight of dish)

$= (W_2 - W_1)$ gm

Volume of sample taken = 50 ml

50 ml sample contains $(W_2 - W_1)$gm of TDS

1000ml of sample contains $(W_2 - W_1) \times \frac{1000}{50} = (W_2 - W_1) \times 20$g of TDS= _____g/l

TDS mg/l = $(W_2 - W_1) \times 20 \times 1000$ = _____

Results and Inference

Expt. No.: 5 **Date:**

Analysis of Temporary Hardness of Waste Water Sample

Introduction

Temporary hardness is a type of water hardness caused by the presence of dissolved carbonate minerals (calcium carbonate and magnesium carbonate). When dissolved, these minerals yield calcium and magnesium cations (Ca^{2+}, Mg^{2+}) and carbonate and bicarbonate anions (CO_3^{2-}, HCO_3^-). The presence of the metal cations makes the water hard. This is also known as carbonate hardness. This causes corrosion in the boilers and other metallic pipes. Hence its determination is also important for agriculture as well as industrial purposes. However, unlike the permanent hardness caused by sulfate and chloride compounds, this "temporary" hardness can be reduced either by boiling the water, or by the addition of lime (calcium hydroxide) through the process of lime softening. Boiling promotes the formation of carbonate from the bicarbonate and precipitates calcium carbonate out of solution, leaving water that is softer upon cooling.

Aim

To determine the temporary hardness in the given waste water sample

Apparatus

100 ml conical flask, 10 ml pipette and 25 ml burette

Reagents

1. Standard sulphuric acid (0.02N): Dilute 0.56 ml of concentrate H_2SO_4 (36N) to one litre with distilled water. (This can be standardized by using 0.05N sodium carbonate solution)
2. Methyl orange indicator (0.5%): Dissolve 0.5 gm dry methyl orange powder in 100 ml of 95% alcohol.
3. Phenolphthalein indicator (0.25%): Dissolve 0.25 gm of pure phenolphthalein powder in 100 ml of 60% alcohol.

Principle

Temporary hardness of waste water is determined by titration of a strong mineral acid to the successive bicarbonate and carbonic acid equivalence points. Phenolphthalein indicator enables the measurement of the alkalinity fraction contributed by the half of the carbonates *i.e,* the carbonates will be converted to bicarbonates which will be known by change in pink colour to colourless. Methyl orange indicator will help in measuring the bicarbonate fractions of alkalinity *i.e.,* both converted from carbonates and original bicarbonates which will be known by change in colour from straw yellow to rose red.

$$H_2SO_4 + CO_3^{2-} \longrightarrow 2HCO_3^- + SO_4^{2-}$$

$$2HCO_3^- + H_2SO_4 \longrightarrow 2H_2O + 2CO_2 + SO_4^{2-} \text{ (rose red)}$$

Procedure

- Pipette 10 ml of given water sample into a 100 ml conical flask
- Dilute it by adding about 25 ml of distilled water
- Add 2-3 drop of phenolphthalein indicator
- If the contents turn to pink colour, it indicates the presence of carbonates
- If pink colour appears, titrate the contents against 0.02N H_2SO_4 till the disappreance of pink colour.
- Note down the titre value with phenolphthalein indicator
- Now add 2-3 drops of methyl orange indicator to the same colorless solution.
- The contents turn to straw yellow colour
- Continue the titration till straw yellow colour changes to rose red colour
- Note down the titre value with methyl orange indicator
- Repeat the titrations till two consecutive and concurrent readings are obtained

Precautions

- Samples must be stored at low temperature and analysed within 24 hours.
- The sample must not be exposed to air or agitated, and even while titrating avoid much shaking to minimize the losses of dissolved gases such as CO_2, H_2S or ammonia.
- Soaps, oily water, suspended solids may interfere. Additional time may be given during titration to get the content colour change. Indicators are used for colourless or slightly coloured samples and potentiometric titration is used in case of dark coloured waste water samples.

Observations

Volume of waste water sample taken = 10ml

Titre value with phenolphthalein indicator

S. No.	Volume of sample ml	Burette reading ml		Titre value ml (Final – Initial)
		Initial	Final	
1	10			
2	10			

Titre value with Methyl Orange indicator

S. No.	Volume of sample ml	Burette reading ml		Titre value ml (Final – Initial)
		Initial	Final	
1	10			
2	10			

Calculations:

Carbonates

1 ml of 1N H_2SO_4 = 60 mg $CaCO_3$

Titre value with phenolphthalein = y ml

Titre value for carbonates = 2y ml

2y ml of 0.02N H_2SO_4 = 2y × 0.02 × 60 (mg $CaCO_3$)

10 ml of sample contains = 2y × 0.02 × 60 (mg $CaCO_3$)

1000 ml (1lit) contains = $\frac{1000}{10} \times 2y \times 0.02 \times 60$ = A mg $CaCO_3$

Carbonates Hardness = A (mg $CaCO_3$) L^{-1}

Bicarbonates

Titre value with methyl orange indicator = x ml

1 ml of 1N H_2SO_4 = 60 mg $CaCO_3$

Titre value for bicarbonates = (x-y) ml

(x-y) ml of 0.02N H_2SO_4 = (x-y) x 0.02 x 60 mg of $CaCO_3$

Volume of sample taken =10ml

∴10 ml of sample contains = (x-y) x 0.02 x 60 mg of $CaCO_3$

1000ml sample contains = $\frac{1000}{10}$ x (x-y) x 0.02 x 60

Bicarbonates Hardness = B mg $CaCO_3$ L^{-1}

Temporary hardness = Carbonates hardness + Bicarbonates hardness
= (A+B) mg $CaCO_3$ L^{-1}

Results and Inference:

Carbonates Hardness (mg $CaCO_3$ L^{-1}) =_____

Bicarbonates Hardness (mg $CaCO_3$ L^{-1}) =_____

Temporary hardness (mg $CaCO_3$ L^{-1}) =_____

Expt. No.: 6 **Date:**

Analysis of Total Hardness of Waste Water Sample

Introduction

Water hardness is defined as the measure of the capacity of the water to precipitate soap. It is caused by compounds of calcium and magnesium, and by a variety of other metals like iron, sulphates *etc.* Water is an excellent solvent and readily dissolves minerals it comes in contact with. Calcium and magnesium dissolved in water are the two most common minerals that make water "hard". Water's hardness is determined by the concentration of multivalent cations in the water. Multivalent cations are cations (positively charged metal complexes) with a charge greater than 1+. Usually, the cations have the charge of 2+. Common cations found in hard water include Ca^{2+} and Mg^{2+}. Soap is precipitated by Ca and Mg of water. Other polyvalent cations are in complex forms with organic matter and their role is minimum. Therefore, total hardness is the sum of Ca and Mg concentration, both expressed as $CaCO_3$ in mg L^{-1}.Rainwater and distilled water are soft, because they also contain a very few ions only. Mineral deposits are formed by ionic reactions resulting in the formation of an insoluble precipitate of calcium carbonate. When hard water is heated, $CaCO_3$ precipitates out; this then clogs pipes and industrial boilers. This leads to malfunction or damage and is expensive to remove. Total hardness of water is usually expressed as concentration of $CaCO_3$ (mg L^{-1}). By determination of Ca & Mg and by calculation hardness of water is determined.

Hardness (mg equivalent of $CaCO_3$ L^{-1})

$$= 2.497\ [(Ca)\ (mg\ L^{-1})] + 4.118\ [(Mg\ (mg\ L^{-1})]$$

The degree of hardness of the water is classified in terms of its calcium carbonate concentration as follows:

Hardness rating	Concentration of Calcium Carbonate (mg/L)
Soft	0 to < 75
Medium hard	75 to < 150
Hard	150 to < 300
Very hard	300 and greater

A. Determination of calcium in waste water sample:

Aim

To determine hardness in terms of concentration of $CaCO_3$ by estimating calcium and magnesium in the given waste water sample by complexometric titration.

Apparatus

Burette, Pipette, china dish and beaker and glass rod

Reagents

1. Ammonium chloride and ammonium hydroxide buffer: dissolve 67.5 g of ammonium chloride in 570 ml conc. ammonium hydroxide and make the volume to one litre with distilled water.
2. Buffer solution (4N NaOH) dissolve 160 g pure sodium hydroxide in distilled water and make the volume to 1 litre with distilled water.
3. Eriochrome black T (EBT) indicator: Dissolve, 0.5 g of the EBT indicator and 4.5 g hydroxylamine hydrochloride in 100 ml of ethanol or methanol.
4. EDTA solution 0.01N: Dissolve 2 g of analytical reagent grade versenate (Ethylene diamine tetraacetate) in distilled water and make the volume to 1 litre. (Standardize the solution with 0.01N calcium solution).
5. Murexide; Also known as ammonium perpurate: Weigh 0.2 gm of ammonium perpurate and 40 gm of potassium sulphate. Mix both the regents thoroughly in a pestle and mortar and keep it as powder in a clean dark coloured bottle.

Principle

EDTA strongly complexes and forms chelates with a number of polyvalent cations such as those of Ca, Mg, Fe, Cu, Mn, Ni, Cd and Zn at different pH values. Based on this principle, Calcium can be estimated by titrating the sample with EDTA maintaining pH 12 as Mg forms insoluble complexes at this pH and only Ca will be titrated. Ca+Mg can be estimated by maintaining pH 10.0 using ammonia buffer as they both form complexes with EDTA at that pH. Indicators namely murexide in case of Ca and EBT in case of Ca+Mg determinations are used to indicate the end point of titration.

Procedure for Ca

- Take 10 ml of water sample into a china dish with a pipette and add few ml of distilled water to increase the volume of solution
- Add 1-2 ml of sodium buffer solution to the contents.
- Add a pinch of murexide indicator till, the contents turn to orange red colour.
- Titrate the contents of conical flask with standard EDTA (0.01N) till orange red changes to purple or purplish violet
- Note down the volume of EDTA run down.

Procedure for Ca+ Mg

- Take 10 ml of water sample into a another china dish with a pipette and add few ml of distilled water to increase the volume of solution
- Add 1-2 ml of buffer solution to the contents.
- Add 2-3 drops of Eriochrome Black T. Stir the contents well with the help of glass rod. The contents turn to wine red colour.
- Titrate the contents of conical flask with standard EDTA (0.01N) till wine red color changes to blue.
- Note down the volume of EDTA run down.

Precautions

- Interfering ions like Fe, Mn, Cu and Zn can be eliminated by converting them into their lower valency states by addition of hydroxylamine hydrochloride.
- It is better if an untitrated sample after colour development is kept by the side of the samples being titrated to distinguish colour with change very precisely. In estimation of calcium do not add more than 25mg of ammonium purpurate as otherwise colour distinction will be very difficult.
- EDTA on storage settles down at the bottom. Before use, either determine its normality again or prepare it fresh.
- Suspended or colloidal organic matter may interfere with the end point. Sample could be evaporated and ignited in muffle furnace at 550^0 C. The residue so obtained could be dissolved in 20 ml HCl, neutralized to pH 7.0 with sodium hydroxide (1N), cooled and used for analysis.

(a) Observations for Ca

S. No	Volume of sample taken	EDTA rundown (ml)		Titre value (ml) (Final-Initial)
		Burette reading Initial	Burette reading Final	
1	10 ml			
2	10 ml			
3	10 ml			

Calculations

1 lit of 1N EDTA	=	20 g of Ca
1 ml of 1N EDTA	=	20 mg of Ca
1 ml of 0.01N EDTA	=	0.2 mg of Ca
Titre value	=	X ml
X ml of 0.01N EDTA	=	X x 0.2mg of Ca
Volume of sample taken	=	10 ml
10 ml of sample contains	=	X × 0.2 mg of Ca

$$\text{Then1000 ml of sample contain} = \frac{1000}{10} \times X \times 0.2 \text{ mg of Ca} = C$$

C= calcium in mg L^{-1}

(b) Observations for Ca + Mg

S. No	Volume of sample taken	EDTA rundown (ml)		Titre value (ml) (Final-Initial)
		Burette reading Initial	Burette reading Final	
1	10 ml			
2	10 ml			
3	10 ml			

Suppose titre value for calcium + magnesium = A ml

Titre value for calcium = X ml

Then titre value for magnesium = (A-X) ml = Y ml

Calculations

1000ml of 1N EDTA = 12 g of Mg

1 ml of 1N EDTA = 12 mg of Mg

1 ml of 0.01N normal EDTA = 0.12 mg of Mg

Titre value = Y ml

Y ml of 0.01N EDTA = 0.12 × Y (mg of Mg)

Volume of sample taken = 10 ml

10 ml of sample contains = (Y × 0.12) mg of Mg

1 lt of sample contains = $\frac{1000}{10} \times Y \times 0.12$ mg of Mg = D

D = magnesium in mg L^{-1}

Total hardness of water (mg equivalent to $CaCO_3$ L^{-1})

= 2.497 {Ca (mg L^{-1}) + 4.118 {Mg (mg L^{-1})}.

=(2.497 × C) + (4.118 × D)

Results and Inference

Expt. No.: 7 & 8 **Date:**

Analysis of Waste Water/Sludge for Heavy Metals

Introduction

In the present situation of water scarcity waste water such as industrial effluents, sewage water are being used for agriculture. Similarly, sludge generated is being either piled up or used in agriculture as a organic source of nutrients. Before making use of waste water/sludge for productive purposes, those have to be analysed for toxic heavy metals to avoid bioaccumulation/biomagnification. Living organisms require heavy metals in trace amounts. However, repeated application of waste water/sludge containing heavy metals may lead to accumulation of heavy metals in toxic levels in crops or animals or human beings which consume the crops produce.

Aim

To determine heavy metals in the given waste water/sludge sample using AAS (Atomic Absorption Spectrophotometer)

Apparatus

1000 ml volumetric flask, 100 ml volumetric flasks, 100 ml conical flasks and funnels, whatman filter paper

Reagents

1. Working standards for Cr, Co, Ni, Cd, Pb: prepare 1000 ppm solutions from standard solution of the above elements and then transfer 0, 0.1, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 ml of 100 ppm solutions into 100 ml volumetric flasks and make up the final volume with double distilled water to obtain 0, 0.1, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 ppm standard solutions.
2. Concentrated HNO_3

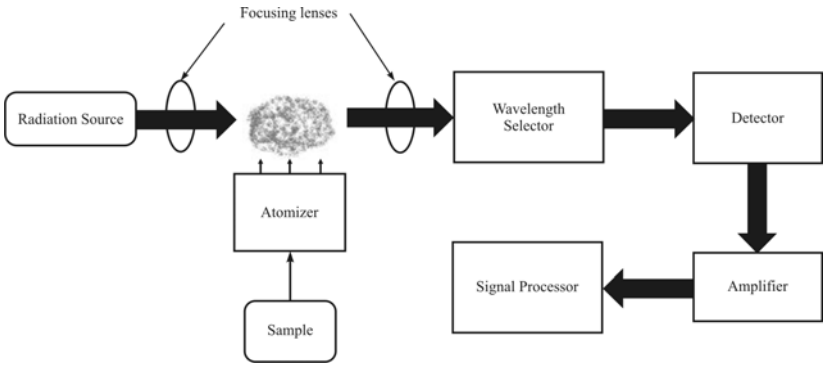
Principle

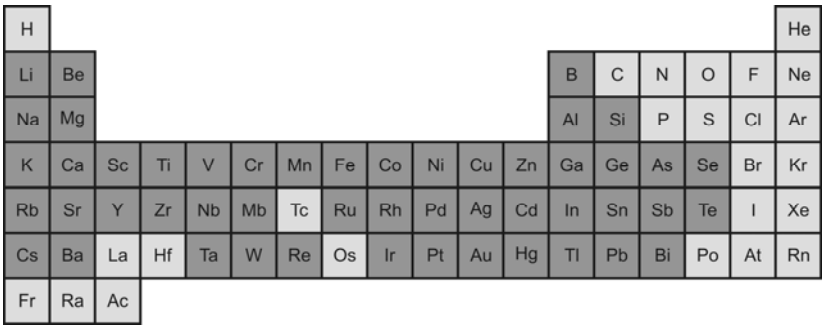
Atomic absorption spectrophotometer is based on the principle that atoms of metallic element normally remain in ground state. These ground state atoms are capable of absorbing radiant energy of their own specific resonance wavelength, which in general is the wavelength of the radiation that the atoms would emit if excited from the ground state. So when radiations of specific wavelength is passed though a flame containing the atoms in question, then part of the light will be absorbed and the absorption of radiation is proportional to the concentration of the atoms of the element. For this the sample in the form of homogenous liquid is aspirated into a flame where “free” atoms of the element to be analysed are created. A light source (hollow cathode lamp) is used to excite the free atoms formed in the flame by the absorbtion of the electromagnetic radiation. The decrease in energy (absorption) in the measured which follows the lambert-beer law, *i.e* the absorbance in proportional to the number of free atoms in the ground state.

Equipment: Atomic absorption spectrophotometer (AAS)

Components of AAS

1. A light source (that generates light at a wave length characteristic to a particular element)
2. A means of aspirating (atomizing an aqueous sample) by nebulizer or burner. The two most common oxidantful combinations used in AAS are air-acetylene and nitrous oxide-acetylene. Generally, flame is used for relatively higher concentration (ppm level) of heavy metals in soil and water, whereas, graphite furnace is used to analyze larger number of elements including mercury arsenic and selenium with greater precision up to parts per billion (ppb) level.
3. A monochromator (separate light of the characteristic wavelength of an element from all other elements)
4. A light sensitive detector, usually a photo multiplier tube, (produces an electric signal proportional to the intensity of light.
5. A means of integrating 'reading' into digital output.





Estimation in waste water

- Take the desired quantity of effluent water sample
- Add 1-2 ml of conc. HNO_3 and keep aside for 10-15 min
- Filter the contents using whatman filter paper. Clear solution is used to note the absorbance in AAS.
- Take the reading of the filtrate in the AAS. It may directly give the content of the metal depending on the model of AAS. Or concentration of each metal in the sample can be calculated by referring to the standard curve.

Estimation in sludge

- Weigh one gram of air-dried sludge sample into acid washed 100ml conical flask and add 20 ml of diacid (HNO_3 : $HClO_4$ in 9:4 ratio) mixture.
- Add 40 ml of distilled water.
- Digest the contents on a hot plate (at about 160^0C) for 2 hours.
- Remove the conical flask from hot plate and cool the contents.
- Make up the contents to 100 ml with double distilled water.
- Filter the contents using whatman No.42 filter paper.
- Take the reading of the filtrate in the AAS. It may directly give the content of the metal depending on the model of AAS. Or concentration of each metal in the sample can be calculated by referring to the standard curve.

Precautions

The apparatus (glass/polyethylene/polypropylene) to be used for the analysis must be thoroughly washed with acidified water and then with deionized water.

Calculations

In waste water sample:

ppm of heavy metal in waste water = (AAS reading in mg L^{-1} × dilution factor)

In sludge sample:

AAS reading = A mg L^{-1}

1 litre or 1000ml contains A mg of heavy metal in question.

100 ml of filtrate contains = A x $\frac{100}{1000}$ mg of heavy metal

1g sludge sample contains…… = A x $\frac{100}{1000}$ mg of heavy metal

(as 100 ml of filtrate is extracted from1g sample)

1000g of sludge sample contains = A x $\frac{100}{1000}$ x 1000 mg of heavy metal

ppm (mg/kg) of heavy metal in sludge sample = (AAS reading in mg L^{-1} × 100)

Results and Inference

Heavy metals content in the given sample

Fe……, Zn ……, Cu……, Mn……,

Pb…, Ni……, Cd……, Co……., Cr……..,

Expt. No.: 9 **Date:**

Determination of Sound Level by Using Sound Level Meter

Introduction

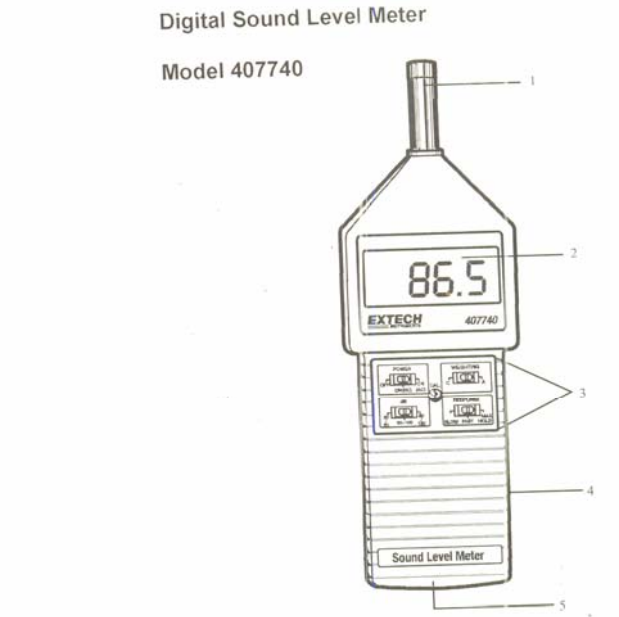
Sound is a form of energy which is emitted by a vibrating body and on reaching the ear causes the sensation of hearing through nerves. Unwanted sound is called noise. The word noise is defined as the 'wrong sound' in the 'wrong place' at 'wrong time'. The differences between sound and noise is often subjective and a matter of personal opinion. What may be considered as music to one person may be noise to another. It is not a substance that can accumulate in the environment like most other pollutants. Noise may not seem as harmful as the contamination of air or water but it effects human health causes irritability, anxiety, lack of concentration and mental fatigue and can contribute to a general deterioration of environmental quality. The intensity of sound is measured in sound pressure levels and common unit of measurement is decibel, Db. The permitted noise level is 125 decibels, as per the Environment (Protection) (second amendment) Rules, 1999.A sound level of 150 dBA or more can physically rupture the human eardrum and >180dB can kill a person.

Aim

To determine the sound in various environments using a digital sound level meter.

Apparatus

Digital sound level meter (Extech 407740) (Figure below)



1. Microphone
2. LCD Display
3. Selector switches for power, Analog Output, A/C Weighting, Response Time, Maximum Hold, and Measurement Ranges
4. Battery Compartment (rear)
5. Analog output jack

Principle

The sound is a form of energy producing longitudinal mechanical waves in matter including solid, liquid and gas (transmitting medium) by oscillation of atoms and molecules of matter. The receiver *i.e.,* microphone absorbs the energy from the transmitting medium and is determined on LCD in terms of decibels.

Procedure

- Turn the power switch to 'ON' to turn the meter on.
- Set the set switch to 'ON' (DC) to configure the analog out put to DC millivolts (1OmV/dB output).
- Use the WEIGHTING selector switch to select 'A' or 'C' frequency weighting. 'A' weighting is used for environmental measurements, OSH regulatory testing, law enforcement and work place design, 'C' weighting is suitable for operational maintenance and analysis of machinery motors, pump engines *etc.*
- Point the microphone towards the source where sound is to be measured.
- Select either fast or slow on 'RESPONSE' switch. Fast is selected to capture sound peaks.
- Select SLOW response with A weighting to the hearing conversation or OSHA related testing.
- The reading displayed is in dB of sound.
- Record maximum dB level selecting 'Max hold' on RESPONSE switch.

Observations

S.No.	Location	Sound in dB
1	In the class room	
2	Inside the plantation area	
3	Open area (without plants)	
4	Ringtone of cell	
5	At the bus stop (while bus is moving)	
6	At the bus stop	
7	Claps	
8		
9		
10		
11		
12		
13		
14		

Results and Inference:

Expt. No.: 10 **Date:**

Estimation Of Species Abundance Of Plants

Introduction

In nature, Organisms belonging to several species can be observed in any given area growing in association with each other. An assemblage of all the interacting population of different species existing in a geographical area is known as a community. It is a complex network of plants, animals and micro organisms. In a community, organisms share the same habit growing in an uniform environment. Communities consists of populations, each of which is a group of interbreeding organisms belonging to the same species. These different species of organisms will impart a structure of community having different populations of organisms. Thus each community is characterized by its growth forms, species diversity, dominance of some species, survival trend *etc*. of nature. Identification of the species which is most populous over the other species and estimating the populations of different species of organisms in that particular area is the estimation of species abundance.

Aim

To study the vegetation by point frame method and determining the frequency of each species present.

Requirements

Point frame apparatus, graph sheet

Procedure

- The apparatus with 10 pins put at a place is one sampling unit. Put the apparatus in random at a number of places (20 or more) in the field.
- Note down each time the various species hit by one or more of the 10 pins and thus, pin is one sampling unit.
- Distribute the various frequencies among five frequency classes and find out the % value in each frequency class.

Observations

Name of the plant	Plants at the following Pin number of the frame										Total no. of individual plants	Frequency %	Density	Abundance
	1	2	3	4	5	6	7	8	9	10				
A														
B														
C														

Calculations

(i) *Frequency:* It is number of sampling unit (as %) in which a particular species occurs.

$$\text{Frequency } (\%) = \frac{\text{No. of sampling units in which the species occurred}}{\text{Total No. sampling units studies}}$$

After determining the percentage frequency of each species, various species are distributed among Raunkier's five frequency classes depending upon their frequency values.

Frequency %	**Frequency class**
0 to 20	A
21 to 40	B
41 to 60	C
61 to 80	D
81 to 100	E

(ii) *Density*: It represents the numerical strength of a species in the community. The number of individuals of the species in any unit area is its density. Density gives an idea of competition.

$$\text{Density} = \frac{\text{Total No. of individuals of the species In all the sampling units}}{\text{Total No. of sampling units studies}}$$

The value obtained is then expressed as number of individuals per unit area.

(iii) *Abundance*: This is the number of individuals of any species per sampling unit of occurrence.

$$\text{Abundance} = \frac{\text{Total No. of individuals of species in all the sampling units}}{\text{No. of sampling units in which the species occurred}}$$

Results and Inference

Expt. No.: 11 **Date:**

Estimation of Respirable and Non-Respirable Dust in Air by Using Dust Sampler

Introduction

The particulate matter in air may occur as solid, liquid and both. The common solid forms are dust (1-200 μ), fumes and smoke while common liquid forms are fog (1-40 μ) and mist (40-500 μ). The particulate matter in air introduced through natural phenomena like wind, volcanic eruptions, pollen and spores, decomposition of organic matter *etc.* or through human activities like industrial processes, mining, burning of fuel *etc.* Air borne particles are considered to be a nuisance to industries requiring clean and aseptic atmosphere like drug and food industry. They may cause diseases like allergic asthma, bronchitis, and even fibrosis of lungs. Dust particles when deposited on the photosynthetic surfaces effect the photosynthesis, growth and yield of agricultural crops. Certain trees are found to be good dust collectors rendering the air clean. Different methods like gravitational method, impaction method, electrostatic precipitation method and filtration method are in vogue to assess suspended particulate matter (SPM) in the air. Broadly particulate matter is categorized into two sizes: >10 microns (non-respirable SPM) and < 10 microns (respirable SPM).

Aim

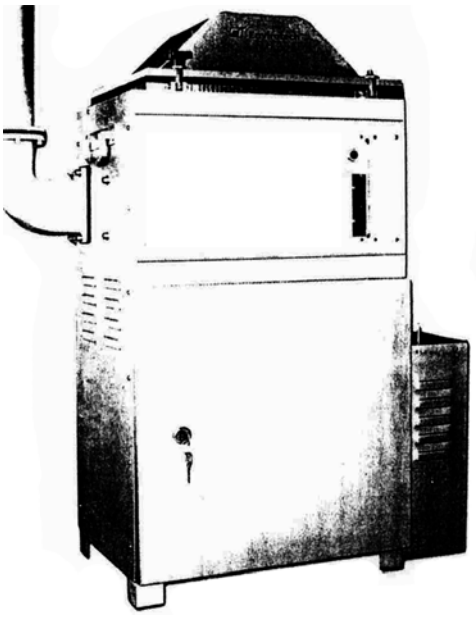
To estimate the amount of respirable and non-respirable suspended particulate matter in the air using high volume sampler.

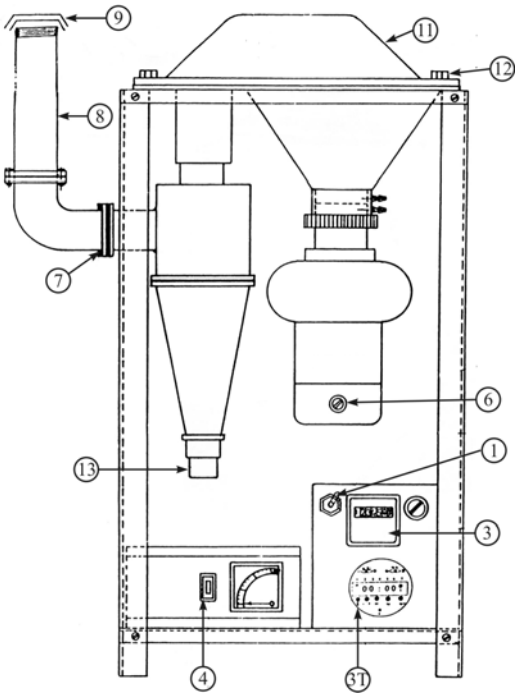
Apparatus

Respirable dust sampler APM 460 , balance

Principle

Ambient air laden with suspended particulates enters the system through the inlet pipe. As the air passes through the cyclone, non respirable dust is separated from the air stream by centrifugal forces acting on the solid particles and is collected in the sampling bottle. The fine dust forming the respirable fraction of the suspended particulate matter passes through the cyclone and is carried by the air stream to the filter paper clamped between the top cover and filter adaptor assembly. The respirable dust is retained by the filter and the carrier air is exhausted from the system through the blower.





Layout of Main parts of APM 460

Procedure

- The air inlet pipe(8) is attached to the cyclone inlet by four socket head cap screws (7) (parts of the dust sampler are marked in the figure above)
- Mount and screw the inlet cover cap (9) on the top of the inlet pipe.
- The manometer assembly (10) is provided to accurately measure the pressure drop across the orifice plate (6) built into the filter adaptor(5) and its scale is graduated to read directly the flow rate of air in m^3/minute.
- Fill the manometer with distilled water with the help of a syringe to the zero mark. Then replace the filling plug screw and tighten it in place.
- **To facilitate the estimation of non respirable dust**: Remove the dust collection bottle (4) at the bottom of the cyclone(2). Use a small paint brush to clean any dust residues at the bottom hole of the cyclone body. Replace a clean pre-weighed dust collection bottle and push it upwards until it rests firmly against the bottom seal ring.
- **To facilitate the estimation of respirable dust:** Check the fibre glass filter for pin holes, particles or other imperpections. Filter with visible imperpections should not be used. Equilibriate the filter in the dessicator for 24 hrs and weight to the nearest milligram. Record the weight and note it as W_3 (do not fold or bend the filter before collection of sample) Open the shelter (11) of the high volume sampler, loosen the wing nuts(12) and remove the face plate from filter holder. Install a pre weighed glass fibre filter in position. Close the roof of the shelter.
- Set the on-off timer to start the sampling for the prescribed time (24 hrs) recording the starting time. After 5 min note down the flow rate.
- Turn switch (1) to ON position and RUN the motor.
- At the end of sampling period, record the length of sampling period and flow rate.
- Note down the weight of the dust collection bottle for calculating the non respirable dust and remove the filter paper and record its final weights as W_1 for estimating respirable dust.

Precautions

- The manometer scale is not linear and it is important to carefully zero the level of the liquid column prior to use without air bubbles.
- Ensure to keep clean sampling bottle initially.
- Ensure that the filter is free of pin holes or any damage.

Observations

Non Respirable Observations

Empty weight of sampling bottle = W_1 gms

Weight of sampling bottle after the sampling time = W_2 gms

Wt of dust collected = ($W_2 - W_1$) gms

Initial flow rate of air = Q_1 (m^3/min)

Finial flow rate of air = Q_2 (m^3/min)

Sampling time in minutes = T

Calculations

Total volume of air sampled in m^3

$$V = \frac{(Q_1 + Q_2) \times T}{2}$$

Mass concentration of suspended particulates in $\mu g/m^3 = \frac{(W_2 - W_1) \times 10^6}{V}$

Respirable Observations:

Weight of filter paper before fixing it in the dust sampler = W_3 gms

Weight of filter paper after the sampling time = W_4 gms

Weight of dust collected = ($W_4 - W3$) gms

Initial flow rate of air = Q_1 (m^3/min)

Finial flow rate of air = Q_2 (m^3/min)

Sampling time in minutes = T

Calculations

Total volume of air sampled in m^3

$$V = \frac{(Q_1 + Q_2) \times T}{2}$$

Mass concentration of suspended particulates in $\mu g/m^3 = \frac{(W_2 - W_1) \times 10^6}{V}$

Expt. No.: 12 **Date:**

Study Of Transpiration And Water Balance In Plants

Introduction

Plant responses to water deficits are often measured under greenhouse or other controlled environmental conditions. Such studies aid in characterization of fundamental physiological responses of plants to drought stress or to identify genetic variability in the genotypes. The identified species could be used either in breeding programmes or in further phenotyping studies.

Aim

To estimate the water requirement of a given genotype / crop and arrive at a balance sheet for the dry matter produced.

Principle

Gravimetric approach makes it possible to maintain precise level of water requirement or stress at whole plant level.

Water stress can be imposed more slowly akin to the natural environment if total plant available water is increased either by using large containers. In such studies the rate at which water stress develops or growth increase depends on the environmental conditions (radiation load, air temperature, humidity) which may vary depending on the water use rates of individual plants. A more consistent treatment that synchronizes well with the experimental units or pots can be achieved by using smaller pots by determining water loss from each pot gravimetrically on a regular basis and replacing part of the transpired water to control the rate of soil dry down. The approach is referred to as gravimetric approach for drought induction studies or assessment of genetic variability and their characterization

Materials

Battery containers or plastic pots, garden soil (soil: sand: manure in the ratio of 2:1:1), mobile weighing device, seed or plant material

Procedure

- Take the empty pot as used for stress imposition experiments
- Fill the pots with soil
- Weigh the pot along with soil and deduct the empty pot weight to obtain the dry soil weight
- Flood the pot with water to saturate soil and leave it for 24 hours to drain of the excess water and consider it as at 100% field capacity (FC)
- Take the pot weight along with pot and deduct the empty pot weight to get the 100% FC soil weight. The dry soil weight is subtracted from the 100% FC soil weight to get the amount of water required to maintain 100% FC.
- Sow the seeds of the crop under irrigation in the pots. Two seedling may be maintained in each pot and watered regularly to maintain moisture level at 100% FC in a poly house.

- After establishment of the seedlings maintain soil at 60% FC *i.e.,* mild stress in pots.
- Take continuous observations at weekly intervals from six leaf stage.
- At each interval the observations include: Record of the amount of water added to maintain 60% FC; pull out the plant and take the dry weight of the entire plant or its component parts.
- Equate the amount of water added and the dry weight increase to know the water balance in plants.

Maintenance of 60% FC is calculated as follows:

100% FC, measured by Keens method = X ml of water

$$60\ \%\ FC = \frac{60\ \% \times X\ ml\ of\ water}{100\%}$$

For example the amount of water required to maintain 100% FC = X ml = 200 ml

$$\text{The amount of water required to maintain 60\% FC} = \frac{60\% \times 200\ ml}{100\ \%} = 120\ ml$$

Observations and Calculations

Pot no (1)	Date (2)	Weight of pot + air dry soil (g) (3)	Amount of water added to bring to 60% FC (ml) (4)	Total Weight of the pot along with plant and soil at 60%FC (g) (5)	Weight of the plant (g) (6)= (5) - (3)+ (4)	Remarks
1						
2						
3						

Discussion

Variation among the genotypes or between crop plants for water requirement could be quantified over a period of time. The genotype which takes little amount of water and puts forth growth or reaches a particular growth stage is considered as a water saver or otherwise water spender. Based on the computation of water requirement and dry matter produced, water balance is arrived at for a genotype. Maintenance of adequate amount of plants / pots would aid in reducing the error and arriving at a water balance sheet for the particular genotype under consideration.

Inference

Expt. No.: 13 **Date:**

Assessment of Chlorophyll Content in Plants

Introduction

Chlorophyll is the essential component for photosynthesis, and occur in chloroplasts as green colored pigment. They are bound loosely to proteins but are readily extracted in organic solvents such as acetone or ether. Chlorophyll content may differ under different conditions of pollution stress and different meteorological conditions, but relatively higher values indicate the relative tolerance of the species to dust pollution. Thus, estimation of chlorophyll is appropriate in evaluation of proper tree species for greenbelts in urban or industrial area. Besides this, as pollution, particularly inert dust, effects/decreases leaf total chlorophyll content and chlorophyll a/chlorophyll b ratio, this is an important parameter to study the effect of dust pollution on agricultural crops. Leaf chlorophyll determination can be done by chemical analysis (Destructive method) or by indirect *in situ* (non-evasive) optical method.

Destructive method

Several chemicals like acetone, ether, Dimethyl Formamide (DMF) and Dimethyl Sulfoxide (DMSO), are used as extractants of chlorophyll. Among these acetone extraction is detailed below as it is (a) simple in use (b) less toxic to skin with a tolerance up to 1000 ppm as compared to other solvents like DMF and DMSO (c) not too procedural as it eliminates requirement of centrifugation (d) And recovery of chlorophyll is 8% more in the acetone incubation method than in the DMSO method.

Aim

Estimation of chlorophyll content in leaves of effected agricultural crops using acetone (destructive method)

Apparatus

Glass mortar and pestle, centrifuge, 100 ml volumetric flask, spectro photometer

Reagent

Diluted analytical grade acetone (80 %) (prechilled).

Principle

Different Chlorophyll absorb light energy differently and each of it has got own absorption peak. Chlorophyll is extracted in 80% acetone and the absorption is read in a spectrophotometer at 645, 652 and 663 nm. Using the absorption coefficient, the amount of chlorophyll is calculated.

Procedure

- Weigh each 1 g of finely cut and well mixed representative sample of effected leaves into a clean glass mortar separately. Avoid mid rib.
- Grind the tissue with a pestle to a fine pulp with the addition of pinch of $CaCO_3$ and 15 ml of 80% acetone. Filter and transfer the filtrate into a 50 ml volumetric flask.
- Grind the residue with 10 ml of 80% acetone, filter and transfer the filtrate to the same volumetric flask.
- Wash the mortar the pestle thoroughly with 80% acetone and collect the clear washings in the same volumetric flask.
- Make up the volume to 50 ml with 80% acetone.
- Read the absorbance of the solution at 645, 652 and 663 nm against the blank (80% acetone).
- If possible repeat the same with a control (unaffected) sample for drawing logical conclusions.

Observations

Absorbance at specific wave lengths = A

Final volume of chlorophyll extract with 80% acetone = V = 50ml

Fresh weight of effected leaf taken for extraction = W gm

Calculations

$$\text{Chlorophyll –a (mg/g of tissue)} = [\ 12.7A_{663} - 2.69A_{645}\ ] \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll –b (mg/g of tissue)} = [\ 22.9A_{645} - 4.68A_{663}] \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll –total (mg/g of tissue)} = [\ A_{652} \times (1000/34.5)] \times \frac{V}{1000 \times W}$$

Result and Inference

Non - destructive method

Nondestructive determination of leaf chlorophyll content permits the measurements of changes in pigments over time for leaves and avoids time-consuming and expensive traditional chlorophyll concentration measurements. These are preferred over methods which involve grinding and centrifugation of tissues as they require a relatively high volume of solvents leading to the lowering of the concentrations of pigments in the final volume, particularly where concentration of chlorophyll is low and also when the material available for sampling is limited. However, for accurate estimation of leaf chlorophyll content the instrument should be calibrated against the chemical analysis for the given plant materials.

Aim

Estimation of chlorophyll content in leaves (effected vs normal) of agricultural crops using SPAD meter

Principle

The SPAD-502 determines the relative amount of chlorophyll present by measuring the absorbance of the leaf in two wavelength regions. The chlorophyll has absorbance peaks in the blue (400-500 nm) and red (600-700 nm) regions, with no transmittance in the near-infrared region. To take advantage of this characteristic of chlorophyll, the SPAD-502 measures the absorbance of the leaf in the red and near-infrared regions. Using these two transmittances, the meter calculates numerical SPAD value which is proportional to the amount of chlorophyll present in the leaf.

Equipment: SPAD-502 meter - is simple to use in the field. It is hand held. Measurements can be made on small leaves also as the measuring area is only 2 mm × 3 mm. It can be used for samples up to 1.2 mm thick.

Procedure

- Perform calibration by pressing on the finger rest to close the measuring head without any sample. Hold it closed until a beep sound.
- Then insert the sample to be measured into the sample slot of the measuring head.
- If measuring a leaf that has many fine veins, take several measurements and average them for best results.
- Press on the finger rest to close the measuring head. Hold it closed until a beep and the SPAD value appears in the display. The measurement will automatically be stored in memory.

Precautions

- Do not use the chlorophyll meter when the plant is under stress because the readings will not be accurate.
- Avoid collecting readings from areas of the field that are not well drained, or from wet leaves because moisture can distort the readings.
- Don't leave the meter in direct sunlight because extreme temperature changes can cause meter readings to fluctuate.

Observations

S.No.	Particulars of sample	SPAD units
1		
2		
3		
4		
5		
6		
7		

Results and Inference

Expt. No.: 14 **Date:**

Visit to *In situ* or *Ex-Situ* Conservation Centre/Social Service Organisation / Environmental Education Centre

Biodiversity or biological diversity is not evenly distributed, rather it varies greatly across the globe as well as within regions. The term Biodiversity was first coined by Walter G. Rosen in 1986. The diversity of all living things depends on temperature, precipitation, altitude, soils, geography and the presence of other species. Biodiversity is crucial to human well being**,** sustainable development and poverty reduction. As defined in convention on Biological diversity signed at Rio De Jenerio (Brazil) in 1992 by 154 countries, the Biodiversity defined as "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic eco-systems and the ecological complexes of which the area part- this include diversity with in species, between species and of ecosystem." A region with a high level of endemic species is called "Hot spot". Hotspots were first named in 1988. While hotspots are spread all over the world, the majority are forest areas and most are located in the tropics. Brazil's Atlantic Forest is considered one such hotspot, containing roughly 20,000 plant species, 1,350 vertebrates, and millions of insects, about half of which occur nowhere else.

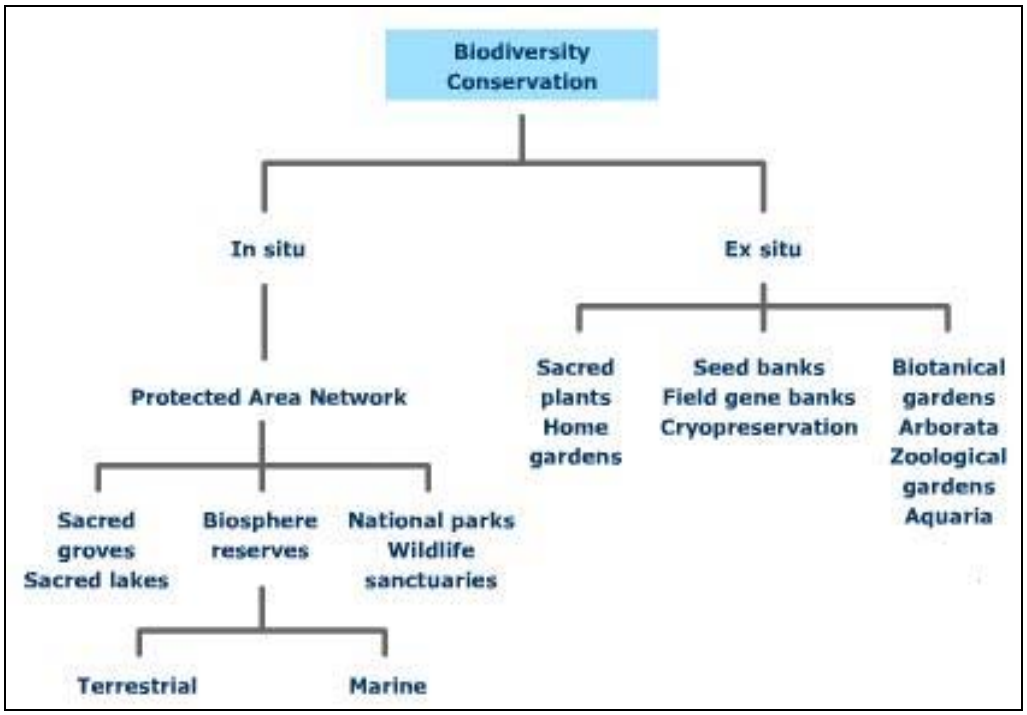
Three kinds of biodiversity are essential to preserve ecological systems and functions.

1. Genetic diversity is a measure of the variety of different versions of the same genes within individual species.

2. Species diversity describes the number of different kinds of organisms within individual communities or ecosystems.

3. Ecological diversity means the richness and complexity of a biological community, including the number of niches, trophic levels, and ecological processes that capture energy, sustain food webs, and recycle materials within this system.

The rapid loss of species we are seeing today is estimated to be between 1000 and 10,000 times higher than the average rate during preceding 65 million years. Loss of biodiversity is due to 1)Natural causes like loss of land mass, low population, low breeding rate, natural disasters *etc* and 2) Anthropogenic causes like habitat modification, overexploitation of selected species, modern agricultural practices, innovation by exotic species, pollution, poaching, global warming and climate change *etc.* Biodiversity can be conserved in two ways *i.e., ex situ* (means "off-site conservation", examples are Zoo, botanical garden, seed or germ plams banks) and *in situ* (means "on-site conservation", examples being National parks and Sanctuaries). The first attempt to bring the biodiversity into the legal frame work was made by way of the biodiversity bill 2000 which was passed by the Lok sabha on 2nd December 2002 and by Rajya Sabha on the December 2002. The Act aims at the conservation of biological resources and associated knowledge as well as facilitating access to them in a sustainable manner and through a just process. For purposes of implementing the objects of the Act it established the National Biodiversity Authority in Chennai.



Experiment

Aim:

Visit to *in situ* or *ex situ* conservation centre/ social service Organization / Environmental Education Centre to bring awareness on conservation of biodiversity

Observations

Write a brief note on the centre you have visited

Expt. No.: 15 **Date:**

Information And Communication Technology (ICT) In Environmental Science

Historically ICT has emerged from the concepts of IT, meaning basically computers and communication technology. ICTs stand for information and communication technologies and are defined, as "diverse set of technological tools and resources used to communicate, and to create, disseminate, store, and manage information." These technologies include computers, software, technology-based recording and processing systems for sound, still and moving images, graphic calculators, robots, the Internet, broadcasting technologies (radio and television), and telephone. Nowadays to improve the efficiency of communication in learner centered environment different technologies are used in combination rather than as the sole delivery mechanism. For instance, the Indira Gandhi National Open University in India combines the use of print, recorded audio and video, broadcast radio and television, and audioconferencing technologies. Online learning or e-learning encompasses learning at all levels, both formal and non-formal, that uses an information network—the Internet, an intranet (LAN) or extranet (WAN)—whether wholly or in part, for course delivery, interaction, evaluation and/or facilitation. Web-based learning is a subset of e-learning and refers to learning using an Internet mainly using a browser (such as Chrome or Firefox or Internet Explorer). Blended learning refers to learning models that combine traditional classroom practice with e-learning solutions. For example, students in a traditional class can be assigned both print-based and online materials, have online mentoring sessions with their teacher through chat, and are subscribed to a class email list.

A good way to think about ICT is to consider all the uses of digital technology that already exist to help individuals, businesses, sciences and organisations use information. For example; Electronic Business, commonly referred to as "eBusiness" or "e-Business", may be defined as the utilisation of information and communication technologies (ICT) in support of all the activities of business. Commerce constitutes the exchange of products and services between businesses, groups and individuals and hence can be seen as one of the essential activities of any business. Hence, electronic commerce or eCommerce focuses on the use of ICT to enable the external activities and relationships of the business with individuals, groups and other businesses. And it was found that the frequency of occurrence of many, information technologies increased at significantly higher rates in the environmental studies than in the general sciences. For instance, one can use ICTs in determining expanding or fluctuating or unexploited research directions by analysing scientific papers published over 10 or 20 years period in all ISI journals in short time stting at one place.

Some of the local specific issues concerning one and all can become a big list. Some of them can be accessed and information about them can be obtained at the following **Uniform Resource Locator (**url). Retrieving pertinent information for the

comparison is sometimes difficult by some inconsistency in the use of keywords and the current lack of semantic oriented tools.

S.No.	URL	Information On
1	http://en.wikipedia.org/wiki/Category:Environment_of_India	*Environment of India* Information is provided on the following topics: Effects of global warming on India, Energising India, Environmental policy of the Government of India, Forestry in India, Indian natural history, Ministry of Environment and Forests (India), Natural disasters in India, Red rain in Kerala, Chipko movement *etc.*
2	http://cicr.nic.in/pop/ap.pdf	Approved Package of Practices for cotton: Andhra Pradesh
3	http://en.wikipedia.org/wiki/Wikipedia:WikiProject_Disaster_management	Disaster management
4	http://www.tropecol.com/pdf/open/PDF_44_1/44110.pdf	Indigenous ecological knowledge, biodiversity and sustainable development in the central Himalayas
5	http://www.fao.org/forestry/en/	Division of forestry of FAO provides information and discusses FAO forestry programmes

Practical exercise

Students to retrieve information from the URL's and submit a small project for record and further evaluation

Expt. No.: 16 **Date:**

Visit to a Local Polluted Site – Observation and Remedial Measures

Pollution can be defined as unwanted or undesirable changes in natural system. Pollutants include solid, liquid or gaseous substances present in greater than natural abundance produced due to human activity, which have a detrimental effect on our environment. An important aspect of notion of pollution is that ecological change actually be demonstrated. If some potentially polluting substance is present at a concentration less than the threshold required to cause a demonstrable ecological change , then the situation would be referred to as contamination, rather than pollution. Polluted sites include urban, rural, agriculture and industrial area. Pollution can affect : Air (smoke and gases from vehicles and industries), Water (sewage, industrial chemical effluents, agricultural pesticides and fertilizers), Soil (chemicals, solid waste from industry, agriculture and urban areas) and Biodiversity (effects on plant and animal life)

Aim

To study the cause and effects of pollution at the site

General observations to be made

Certain key questions related to the polluted site are given below. Explore the site to answer the questions about the area you have visited

- About the site? The type of land or water use in the polluted area, its geographical characteristics, who uses the area, who owns it.
    - Rural – agricultural area, water body, industrial area
    - urban – solid waste management site, industrial area , water body
- Map the area to be studied.
- Identifying what is being polluted – air, water, soil; the cause(s) of pollution and the polluting agent(s)

  Solid waste- garbage dump, polluted water at a river or lake, effluents, smoke coming out of an industry area, etc
- Explore the reasons for pollution. Observe and document the components in the garbage/the polluted water body/industrial chimneys.
- Track the path of pollutants/pollution with the relevant past land history in relation to possible land contamination (e.g. accident records, change of land use, reclamation of polluted seabed and any other relevant information);
- Assess the extent of pollution- severe / moderate/ slight / nil, to: the air, water, soil, biodiversity
- What are the effects of the pollutant?

  Assess from literature, the health aspects associated with the pollutant.

  Ask local residents about its effects on their lives.
- What actions can you suggest to get the pollution reduced?
- Make a report of the above findings.

Write a brief note on the site you have visited

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2. HLS Tandon *Methods of Analysis of Soils, Plants, Waters and Fertilisers*, published by Wadha International, B-193, Okhla Phase- I, New Delhi – 110020.