ORIGINAL RESEARCH ARTICLE

SEA WATER NUTRIENT ANALYSIS METHODS

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ABSTRACT:

The quality of coastal water is dependent on its nutrient concentration and other indices. Coastal water receives waste from different sources and can result in harmful algal blooms. Anthropogenic stress inducers on fresh water bodies ultimately reach the marine environment and pollute the coastal waters. This chapter deals with basic nutrient analysis methodology of marine samples. The methods discussed have been standardized in our laboratory.

KEYWORDS: Sea water, nutrient analysis.

INTRODUCTION:

The quality of coastal water is dependent on its nutrient concentration and other indices. Coastal water receives waste from different sources and can result in harmful algal blooms. Anthropogenic stress inducers on fresh water bodies ultimately reach the marine environment and pollute the coastal waters. The primary nutrients which act as regulators of good

health of coastal environment are Nitrate and Phosphate. These Nutrients in Sea water are highly unstable and in excess concentration they induce flourishing of harmful marine plants and animals. Due to their sensitivity, marine water samples for all nutrients should be maximally analysed within six hours after collection [2]. This chapter deals with basic Nutrient analysis methodology of marine samples. The methods discussed have been standardized in our laboratory.

The mode of preservation:

Phosphate	Refrigeration at -4°C
Sulphate	Refrigeration at -4°C
Silicate	Refrigeration at -4°C
Nitrate	Refrigeration at -4°C
Nitrite	Refrigeration at -4°C

I. DETERMINATION OF SULPHATE IN SEA WATER:

PRINCIPLE:

The turbidimetric method of measuring sulphate is based on the fact that sulphate is precipitated as barium sulphate by addition of barium chloride and this tendency is enhanced in presence of sodium chloride, hydrochloric acid and glycerol [4].

$SO_4^{2-} + BaCl_2 \longrightarrow BaSO_4$

The absorbance of the barium sulphates formed is measured by a spectrophotometer at 420 nm and the sulphate ion concentration is determined by comparison of the reading with a standard curve.

MATERIALS REQUIRED:

APPARATUS REQUIRED:

UV- Visible Spectrophotometer, Magnetic stirrer, Volumetric flask, Volumetric pipette, Beaker, Glass rod, Measuring Cylinder, Spatula, Pipette, Wash Bottle, Tissue Paper

CHEMICALS REQUIRED:

Isopropyl Alcohol, Glycerol, 35% Hydrochloric acid, Sodium Chloride, Barium Chloride, Sodium Sulphate, Distilled Water

PROCEDURE:

PREPARATION OF REAGENTS:

Conditioning reagent

Take 250 ml volumetric flask and transfer 25ml of glycerol, followed by 15ml of 35% HCl. To the same flask add exactly 50ml of 95% Isopropyl alcohol and mix well. Accurately weigh 37.5g Sodium chloride and dissolve it in distilled water separately. Then mix all the contents and make up the final volume to 250ml.

Standard Sulphate solution

Dissolve 0.1479g of anhydrous Na_2SO_4 (Sodium sulphate) in distilled water and make up the final volume to 1000ml. The stock solution contains 100mg/l of SO_4^{2-} (Sulphate).

Preparation of Standard for Standard curve, Blank and sample analysis

Take 10ml, 20ml, 30ml, 40ml and 50ml of standard stock Sulphate solution in five different volumetric flasks. Then adjust the volume to 100ml by adding distilled water. These solutions contain 10mg/l, 20mg/l, 30mg/l, 40mg/l and 50mg/l of sulphate respectively. To each flask add 5ml

of conditioning reagent and stir the sample with a magnetic stirrer. Add 2g of $BaCl_2$ during stirring and continue this for 1minute. Measure the absorbance at 420 nm.

For blank take 100ml of distilled water, add 5ml of conditioning reagent and 2g of $BaCl_2$ during stirring and continue this for 1minute. Measure the absorbance at 420 nm.

For sample analysis filter the sample through Whatman No. 1 filter paper. Take 20ml of the filtered sample in a volumetric flask and then make up the final volume to 100ml by adding distilled water. To this add 5ml of conditioning reagent and 2g of $BaCl_2$ as mentioned above. Then measure the absorbance at 420nm.Unknown concentration of Sulphate can be calculated from the Standard curve.

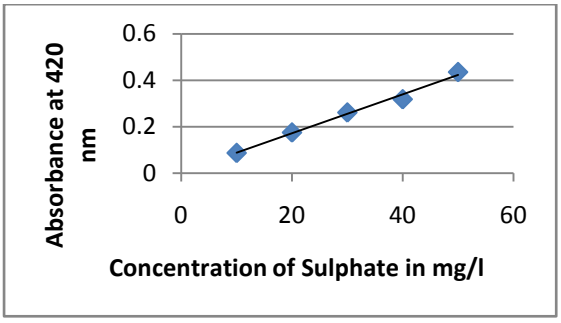

Figure 1. Standard curve of Sulphate

II. DETERMINATION OF SILICATE IN SEA WATER:

PRINCIPLE:

Sea water reaction with molybdate results in the formation of silicomolybdate, phosphomolybdate and arsenomolybdate complex. Metol and oxalic acid are used as reducing solution capable of reducing the silicomolybdate complex to give a blue reduction compound. The reducing solution also decomposes any phosphomolybdate or arsenomolybdate to prevent interference from phosphate and arsenate [3].

MATERIALS REQUIRED:

APPARATUS REQUIRED:

UV- Visible Spectrophotometer, Volumetric flask, Volumetric pipette, Beaker, Glass rod, Measuring Cylinder, Spatula, Pipette, Wash Bottle, Tissue Paper

CHEMICALS REQUIRED:

Ammonium molybdate, 35% HCl, Metol Sulphite, Na_2SO_3, Oxalic acid, H_2SO_4, Sodium silicofluoride (Na_2SiF_6)

PROCEDURE:

PREPARATION OF REAGENTS:

Ammonium molybdate

Dissolve 0.4g of ammonium molybdate in 30ml of distilled water and add 1.2ml of 35% HCl . Make up the final volume to 50ml and keep it in a polyethylene bottle.

Metol sulphite

Dissolve 0.48g of Na_2SO_3 in 40ml of distilled water, to this add 0.8g of metol. After dissolving, filter the solution through Whatman No-1 filter paper and store in clean glass stoppered bottle.

Oxalic acid

Dissolve 2g of oxalic acid in 20ml distilled water and store in a clean glass bottle.

50% H_2SO_4

Take 25ml of Conc. H_2SO_4 and add 25ml of distilled water slowly to make up the final volume to 50ml.

Reducing reagent

Mix 25ml of metol sulphite solution with 15ml of oxalic acid. Slowly add 15ml of 50% H_2SO_4. Then make up the final volume of the mixture to 75ml with distilled water.

Standard Silicate solution

Take 0.960g of dried Na_2SiF_6 in distilled water and make up the volume to 1000ml in a volumetric flask. This solution contains 5000µg/l of silicate. Store this solution in a polyethylene bottle. This is solution 'A'.

Dilute 10ml of 'A' to 100ml with distilled water. This is solution 'B'.

Dilute 10ml of 'B' to 250ml with distilled water. This is stock solution 'C' containing 20µg/l silicate.

Preparation of Standard for Standard curve, Blank and sample analysis

From the stock solution 'C' pipette 10ml, 20ml, 40ml and 60ml solution in four different volumetric flask and make up the volume to 100ml with distilled water. This working solution contains 2µg/l, 4µg/l, 8µg/l and 12µg/l of silicate respectively. Take 4ml of ammonium molybdate in 8 different test tubes of 25 ml capacity. Pipette out 10ml of each concentration of silicate working solution in duplicate and add to the test tubes containing Ammonium molybdate. Add 6ml of reducing reagent each to the tubes containing 4 ml of ammonium molybdate and 10 ml of varying concentration of silicate working solution (2µg/l, 4µg/l, 8µg/l and 12µg/l). Mix the tubes and keep it at room temperature for 1 hour. Measure the absorbance at 810nm after one hour.

For blank take 10ml of distilled water and add to 4ml of ammonium molybdate along with 6ml of reducing reagent. Then take the absorbance after one hour incubation.

For sample analysis, filter the sample through Whatman filter paper No.1. Add 10ml of the filtered sample to 4ml ammonium molybdate solution. To this add 6ml reducing reagent and keep it at room temperature for one hour. Measure the absorbance at 810nm. Calculate the concentration of silicate from the Standard curve.

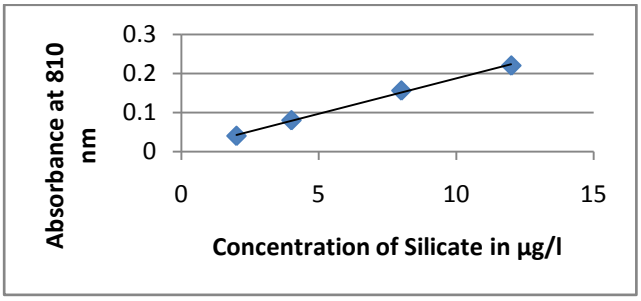

Figure 2. Standard curve of Silicate

III. DETERMINATION OF PHOSPHATE IN SEA WATER

A. DETERMINATION OF ORTHOPHOSPHATE:

PRINCIPLE:

The Orthophosphate reacts with acidified ammonium molybdate solution

and forms molybdophosphoric acid, which is then reduced to blue complex in the presence of stannous chloride[2],[3].

MATERIALS REQUIRED:

APPARATUS REQUIRED:

UV- Visible Spectrophotometer, Water bath, Volumetric flask, Beaker, Glass rod, Measuring Cylinder, Spatula, Pipette, Wash Bottle, Tissue Paper

CHEMICALS REQUIRED:

Ammonium molybdate, Con. H_2SO_4, Stannous chloride, Glycerol, Potassium dihydrogen phosphate, Distilled water

B. DETERMINATION OF TOTAL PHOSPHATE:

PRINCIPLE:

All forms of phosphorous i.e. soluble reactive phosphate, polyphosphate, organic phosphate are converted to soluble inorganic orthophosphate by digestion with perchloric acid and oxidation with sodium hydroxide. The phosphate thus released, can be determined colorimetrically by following the Orthophosphate determination method[2],[3].

MATERIALS REQUIRED:

APPARATUS REQUIRED:

UV- Visible Spectrophotometer, Water bath, Volumetric flask, Beaker, Glass rod, Measuring Cylinder, Spatula, Pipette, Wash Bottle, Tissue Paper

CHEMICALS REQUIRED:

Perchloric acid, Phenolphthalein, Ethanol, NaOH, Ammonium molybdate, Con. H_2SO_4, Stannous chloride, Glycerol, Potassium dihydrogen phosphate, Distilled water

PROCEDURE:

PREPARATION OF REAGENTS:

Perchloric acid about 60% - 70%

Phenolphthalein indicator

Dissolve 0.5g phenolphthalein in 150ml of 95% ethanol and 50ml of distilled water. To the same solution add 0.02N NaOH drop wise till the appearance of faint pink colour.

8% NaOH

Dissolve 8g NaOH in 100ml of distilled water.

Ammonium molybdate

a) Dissolve 25g of ammonium molybdate in 75 ml of distilled water and make up the total volume to 175ml using distilled water (**Sol – A**).
b) Take 280ml of Conc.H_2SO_4 and slowly add 400ml of distilled water. Allow it to cool down (**Sol- B**).

Mix the two solutions **'A' and 'B'** and dilute to 1L in a volumetric flask.

Stannous chloride

Dissolve 2.5g of stannous chloride in 100ml glycerol by heating on a water bath for rapid dissolution.

Standard Phosphate solution

Dissolve 4.388g dried anhydrous potassium dihydrogen phosphate in distilled water and make up the final volume to 1000 ml. Dilute this solution

hundred times. This is standard phosphate stock solution containing 10mg/l phosphate.

Preparation of Standard for Standard curve, Blank and sample analysis

Standard working solution preparation for standard curve is same as that of Orthophosphate.

For blank and sample analysis take 25ml of distilled water and 25ml of filtered sample respectively. Then evaporate it to complete dryness at 70°C. After cooling add 1ml perchloric acid and again evaporate. Allow the residue to cool and after cooling add 100ml of distilled water followed by 1 drop of phenolphthalein indicator. Then titrate the sample against 8% NaOH drop wise till the appearance of pink colour. From this solution pipette out 25ml sample and add 1ml ammonium molybdate followed by 3 drops of stannous chloride. Measure the absorbance at 690nm after 10-15 minutes and calculate the unknown concentration from standard curve.

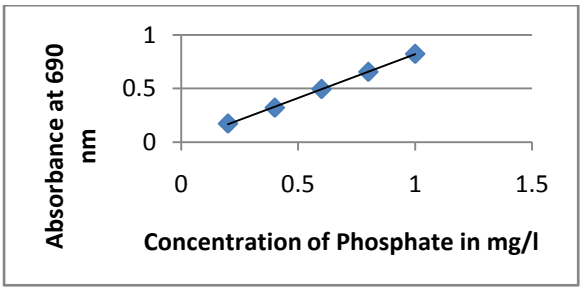

Figure 3. Standard curve of Phosphate (Orthophosphate and Total phosphate)

IV. DETERMINATION OF NITRITE IN SEA WATER:

PRINCIPLE:

In acid solution, the nitrite yields nitrous acid, which diazotises the sulphanilamide. The diazonium salt when reacts with aromatic amine, N-1, naphthyl ethylenediamine dihydrochloride forms a red dye, which is measured spectrophotometrically[1],[2],[3].

MATERIALS REQUIRED:

APPARATUS REQUIRED:

UV- Visible Spectrophotometer, Water bath, Volumetric flask, Beaker, Glass rod, Measuring Cylinder, Spatula, Pipette, Wash Bottle, Tissue Paper

CHEMICALS REQUIRED:

Sulphanilamide, 35% HCl, NEDA (N-1, naphthyl ethylenediamine dihydrochloride), Sodium nitrite, Distilled water

PROCEDURE:

PREPARATION OF REAGENTS:

Sulphanilamide solution

Add 50ml of 35% Conc. HCl to 400ml of distilled water, to this add 5g of sulphanilamide reagent and make up the final volume to 500ml.

NEDA

Dissolve 0.5g of NEDA in 500ml of distilled water. Keep the reagent in a dark bottle.

Standard Nitrite solution

Dissolve 0.345g of sodium nitrite in 1000ml of distilled water. **This is primary standard 'A'.**

Dilute 10ml of 'A' to 1L with distilled water. This is **secondary standard 'B'.**

Dilute 10ml of 'B' to 100ml with distilled water. **This is the final stock solution containing .00500034 mM/l nitrite.**

Preparation of Standard for Standard curve, Blank and sample analysis

Take 2.5ml, 5ml, 10ml, 15ml, 25ml and 50ml of the stock solution in different volumetric flasks in duplicates. Then make up the volume to 100ml with distilled water. This working solution contains 0.000125mM/l, 0.00025mM/l, 0.0005mM/l, 0.00075mM/l, 0.00125mM/l and 0.0025mM/l nitrite respectively. To this add 1ml of sulphanilamide, 1ml of NEDA after 10 minutes. Incubate the solution for 10-15 minutes. Measure the absorbance at 543nm.

For blank and sample analysis, pipette out 25ml of distilled water and 25ml of filtered sample (Whatman No. 1 Filter paper) respectively. To this add 0.5ml sulphanilamide and wait for 10 minute. Then add 0.5ml NEDA and again wait for 10-15 minute. Measure the absorbance at 543 nm and calculate the unknown concentration of nitrite from the standard curve.

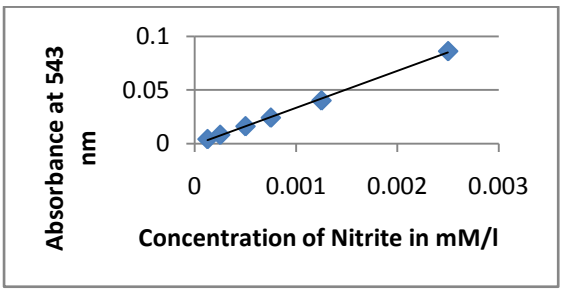

Figure 4. Standard curve of Nitrite

V. DETERMINATION OF NITRATE IN SEA WATER

PRINCIPLE:

Nitrate in sea water is reduced to nitrite by heterogenous reduction through cadmium granules. Nitrite is determined by diazotising with sulphanilamide and coupling with N-1, naphthyl ethylenediamine dihydrochloride. The coupling reaction using NEDA gives a coloured azo dye [1],[2],[3].

MATERIALS REQUIRED:

APPARATUS REQUIRED:

UV- Visible Spectrophotometer, Water bath, Volumetric flask, Beaker, Glass rod, Measuring Cylinder, Spatula, Pipette, Wash Bottle, Tissue Paper

CHEMICALS REQUIRED:

Sulphanilamide, 35% HCl, NEDA (N-1, naphthyl ethylenediamine dihydrochloride), Potassium nitrate, Ammonium chloride, Ammonia solution(32%), Copper sulphate, Distilled water

PROCEDURE:

PREPARATION OF REAGENTS:

Ammonium chloride buffer

Disssolve 5g of ammonium chloride in distilled water and make up the final volume to 500ml. To this add 0.7ml of ammonia solution (32%) to adjust pH level.

2% copper sulphate solution

Dissolve 2g of copper sulphate in 100ml of distilled water. This is REQUIRED

for washing of cadmium granule before packing the column.

Sulphanilamide solution

Add 50ml of 35% Conc. HCl to 400ml of distilled water. To this add 5g of sulphanilamide reagent and make up the final volume to 500ml.

NEDA

Dissolve 0.5g of NEDA in 500ml of distilled water. Keep the reagent in a dark bottle.

Standard Nitrate solution

Dissolve 0.1011g dry potassium nitrate in 100ml of distilled water. Pipette 1ml of Standard Nitrate solution and dilute it to 100 ml. This is **stock nitrate solution containing 100µM/l nitrate.**

Preparation of Standard for Standard curve, Blank and sample analysis

Take 2.5ml, 5ml, 7.5ml, 10ml and 12.5ml of stock solution in different volumetric flask. Then adjust the final volume to 50ml and divide the solution in to two sets of 25 ml each (25+25). This is the working solution contains 5µM/l, 10µM/l, 15µM/l, 20µM/l and 25µM/l nitrate respectively. To this add 0.5ml of sulphanilamide and after 10 minutes add 0.5ml of NEDA. Take the absorbance at 543nm after 10-15 minutes of incubation.

For blank and sample analysis take 50ml of distilled water and 50ml of filtered sample respectively (Whatman No. 1 filter paper). Release each 50ml of sample and 50ml of ammonium chloride buffer through the cadmium reduction column. Discard the first 25ml and collect the next 50ml as 25ml + 25ml in two different conical flasks. Add 0.5ml of sulphanilamide to this and wait for 10 minutes. Again add 0.5ml of NEDA and leave for 10-15 minute incubation. After incubation measure the absorbance at 543nm and calculate the unknown concentration of nitrate from the standard curve.

Column precaution

Cadmium column can effectively process 100 samples without change of cadmium granules. After processing 100 samples the granules should be replaced to minimize error. The upper layer of column should always be submerged with ammonium chloride solution. The column should have slow flow rate. Addition of ammonium chloride increases the life of the column.

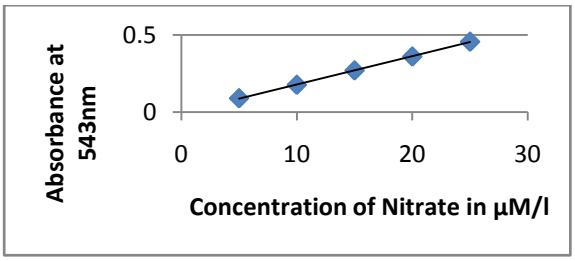

Figure 5. Standard curve of Nitrate

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