Tier 2 Field Protocols

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Randomly generated station coordinates within hexagons are accessed from a GPS device which enables precise location of the station and a reference point for sampling. Stations are approached slowly to ensure that the deceleration of the boat does not create a wake and re-suspend sediments into the water column. All hydrographic and water column measurements are collected while the vessel is set to drift over the station to minimize interference from sediment plumes generated from physical disturbances of the bottom. Sonde deployment, water transparency and water sample collection must occur within 30 m of the station location.

Measurements are made in the following order: (1) PAR, (2) visibility, (3) hydrography, (4) TSS, (5) depth, (6) percent coverage, (7) canopy height, and (8) blade samples for C:N:P ratios.

1) While taking measurements, be careful not to disturb the sediment. Lower the light sensor gently into the water taking care to prevent the vessel from shading the sensor. Keep the frame perpendicular to the water surface. The height (or distance) between the two sensors (normally 0.5 m) must be measured and recorded before deployment.

2) When using a secchi disk for visibility measurements, be sure to remove your sunglasses and prevent the vessel from shading the disk. Denote the diameter of the secchi unless otherwise recorded.

3) Lower the sonde so that the instrument probes are completely submerged. At stations deeper than 1 m, take additional measurements above the sediment-water interface. Avoid agitating the seagrasses or sediments since this can re-suspend microalgae and compromise the accuracy of the *in situ* chlorophyll probe.

4) For TSS, rinse bottles and lids three times with sample water before collecting the sample. If you have applied sunscreen, wear gloves to prevent contamination of sample.

5) Measure depth using the Precision Depth Recorder (i.e. PVC marked in 10 cm segments). Avoid allowing the PVC pole to sink into soft sediments.

Once the hydrographic data and water samples are collected, the boat is anchored on the station coordinates. Deployment of the anchor requires care to minimize disturbance of the sediments.

The four corners of the boat (port-stern, port-bow, starboard-bow, and starboard-stern) are used as seagrass sample locations. Measurements are taken approximately 2-4 meters from the boat by tossing a 0.25 m^2 quadrat into the water.

6) Percent coverage is estimated within the 0.25 m^2 quadrat. The quadrat is used as a reference to visually estimate seagrass cover.

7) Within each quadrat, five canopy height measurements are made for seagrass species present.

8) Collect samples of *Thalassia* and *Halodule* blades for C:N:P analysis by manually snapping the seagrass shoot from the rhizome and keeping the sheath intact. Rinse sediment from tissue samples in the field; epiphytes will be removed in the lab. Be careful not to contaminate samples with sunscreen, lotion, etc. Roll the seagrass blades (minimum of 6 for *Thalassia* and 15 for *Halodule*) and place in a labeled Whirl-pak bag, removing all air, sealing, and placing on ice.

Record meteorological and environmental information on field data entry forms (e.g. cloud cover, wind speed and direction, any problems, seagrass characteristics, etc.).

Percent Surface Irradiance (% SI) and Light Attenuation (*k*)

Developed by: Kenneth Dunton and Kimberly Jackson
Last Revised: December 2009
Approved by: EPA (2002) and TPWD (2010)

Field Procedures

Measurements of percent surface irradiance (% SI) and the diffuse light attenuation coefficient (*k*) are made from simultaneous measurements of surface (ambient) and underwater irradiance.

Surface measurements of photosynthetically active radiation (PAR = ca. 400 to 700 nm wavelength) are collected using an LI-190SA quantum sensor that provides input to a LI-1000 datalogger (LI-COR Inc., Lincoln, Nebraska, USA). Underwater measurements are made using a LI-192SA or LI-193SA spherical quantum sensor.

Measurements of % SI and *k* are based on three or more replicate determinations of instantaneous PAR collected by the surface and underwater sensors and recorded by the LI-1000 datalogger. Care is taken to reduce extraneous sources of reflected light (from boats or clothing).

Calculations

Percent surface irradiance available at the seagrass canopy is calculated as follows:

$$\% \text{ SI} = (I_z/I_0) \times 100$$

where I_z and I_0 are irradiance (µmol photons $m^{-2}sec^{-1}$) at depth z (meters) and at the surface, respectively.

Light attenuation is calculated using the transformed Beer-Lambert equation:

$$k_d = -[\ln(I_z/I_0)]/z$$

where *k* is the attenuation coefficient (m^{-1}) and I_z and I_0 are irradiance (µmol photons m^{-2} sec^{-1}) at depth z (meters) and at the surface, respectively.

Total Suspended Solids (TSS)

Developed by: Kenneth Dunton and Kimberly Jackson
Adapted from: EPA METHOD #: 160.2
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Scope and Application

This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes. The practical range of the determination is 4 mg/L to 20,000 mg/L.

Summary of Method

A well-mixed sample is filtered through a glass fiber filter, and the residue retained on the filter is dried to constant weight at 103-105°C.

Sample Handling and Preservation

Non-representative particulates such as leaves, sticks, fish, and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result. Preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to 4°C, to minimize microbiological decomposition of solids, is recommended.

Interferences

Filtration apparatus, filter material, pre-washing, post-washing, and drying temperature are specified because these variables have been shown to affect the results. Samples high in Filterable Residue (dissolved solids), such as saline waters, brines and some wastes, may be subject to a positive interference. Care must be taken in selecting the filtering apparatus so that washing of the filter and any dissolved solids in the filter minimizes this potential interference.

Laboratory Procedures

1) Dry new filters at 60°C in oven prior to use.

2) Weigh filter immediately before use. After weighing, handle the filter or crucible/filter with forceps or tongs only.

3) Place the glass fiber filter (i.e. Glass fiber filter discs, without organic binder, such as Millipore AP-40, Reeves Angel 934-AH, Gelman type A/E, or equivalent; Our lab uses 47 mm GF/F 0.7 micron retention) on the membrane filter apparatus. *NOTE: Because of the physical nature of glass fiber filters, the absolute pore size cannot be controlled or measured. Terms such as "pore size", "collection efficiencies" and "effective retention" are used to define this property in glass fiber filters.*

4) For a 47 mm diameter filter, filter 100 mL of sample. If weight of captured residue is less than 1.0 mg, the sample volume must be increased to provide at least 1.0 mg of residue. If other filter diameters are used, start with a sample volume equal to 7 mL/cm of filter area and collect at least a weight of residue proportional to the 1.0 mg state above. *NOTE: If filtering clear pristine water, start with 1 L. If filtering turbid water start with 100 mL.*

NOTE: If during filtration of this initial volume the filtration rate drops rapidly, or if filtration time exceeds 5 to 10 minutes, the following scheme is recommended: Use an unweighed glass fiber filter affixed in the filter assembly. Add a known volume of sample to the filter funnel and record the time elapsed after selected volumes have passed through the filter. Twenty-five mL increments for timing are suggested. Continue to record the time and volume increments until filtration rate drops rapidly. Add additional sample if the filter funnel volume is inadequate to reach a reduced rate. Plot the observed time versus volume filtered. Select the proper filtration volume as that just short of the time a significant change in filtration rate occurred.

5) Assemble the filtering apparatus and begin suction.

6) Shake the sample vigorously and quantitatively transfer the predetermined sample volume selected to the filter using a graduated cylinder. Pour into funnel.

7) Remove all traces of water by continuing to apply vacuum after sample has passed through.

8) With suction on, wash the graduated cylinder, filter, non-filterable residue and filter funnel wall with three portions of distilled water allowing complete drainage between washing. Remove all traces of water by continuing to apply vacuum after water has passed through.

NOTE: Total volume of distilled rinse water used should equal no less than 50 mLs following complete filtration of sample volume.

9) Carefully remove the filter from the filter support.

10) Dry at least one hour at 103-105°C. Overnight drying ensures accurate filter weight.

11) Cool in a desiccator and weigh.

12) Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg).

Calculations

TSS (mg/L) is calculated as follows:

$$1000 \times (A-B) \times (1000/C) = TSS$$

where A = weight of filter (or filter and crucible) + residue (mg), B = weight of filter (or filter and crucible) (mg), and C = amount of sample filtered (mL).

Sediment Grain Size

Developed by: Kenneth Dunton and Kimberly Jackson
Adapted from: Rick Kalke, Paul Carangelo and Dr. E.W. Behrens
Last updated: December 2009
Approved by: EPA (2002) and TPWD (2010)

Field Procedures

Three replicate sediment samples are collected at each station using a plastic syringe (2.5 cm diameter, 10 cm length) driven up to 10 cm into the seafloor (depth is determined by sediment compactness). These samples are placed in pre-labeled Whirl-pak bags and immediately placed on ice for transport to the lab.

Summary of Method

Samples are either immediately frozen or processed upon return to the lab. If samples are frozen, the samples will be thawed overnight in a refrigerator prior to processing. To determine sediment grain size, sand/silt/clay ratios are determined following the methods of Folk (1964). Percent contribution by weight is measured for four components: rubble, sand, silt, and clay. A 20 mL sediment sample is mixed with 100 mL of 3% hydrogen peroxide and 75 mL of de-ionized water to digest organic material in the sample. The sample is then wet sieved through a 62 µm mesh stainless steel screen using a vacuum pump and a Millipore Hydrosol SST filter holder to separate rubble and sand from silt and clay. After drying, the rubble and sand will be separated on a 250 µm screen. The silt and clay fractions will be measured using pipette analysis. Briefly, the settling velocity will be used to classify the particles and to determine the percent composition of each fraction, based on weight.

Laboratory Procedures

1) Wash all glassware with detergent (Alconox) and rinse with distilled or deionized water.
2) Extract 20 cc of homogenized sample using a wide-mouth syringe, or fill syringe using a spatula.
3) Place sample in a labeled beaker and add 100 mL 3% hydrogen peroxide. Mix with a rubber policeman and let sit until liquid is clear (several days). *NOTE: This step is to digest the organics in the sample.*
4) Weigh labeled aluminum pans and 62 µm stainless steel filters; one each for each sediment sample.
5) Decant excess hydrogen peroxide (as much as possible without stirring up the sample). Add approximately 100 mL deionized water to the sample; stir and filter using a vacuum pump and a Millipore Hydrosol SST Filter Holder with 62 µm screen. Repeat two more times or until water is more or less clear. Dump the remaining sand and rubble onto the filter; rinse beaker and rubber policeman. *NOTE: Do not use more than 900 mL water in this step.*

6) Place sand and screen in weighing pan and dry at 100-130°C for at least 24 hours.

7) Pour the filtrate in a 1 L graduated cylinder. Add 10 mL 10% Calgon dispersant and dilute to 1 L. After all samples reach room temperature, take water temperature and refer to temperature/fall distance table to determine the length of time between the first and second withdrawals.

8) Weigh and label two beakers (A and B) for each sample.

9) Stir the samples uniformly, working with one sample at a time. Twenty seconds after stirring, insert a pipette to a depth of 20 cm and withdraw 20 mL of the suspension. Put first withdrawal in the pre-weighed A beaker. Rinse pipette with deionized water into A beaker. Proceed with other samples.

10) Second 20 mL withdrawal is taken from a depth of 10 cm at the time indicated on the temperature/fall distance table and placed in the pre-weighed B beaker. Rinse pipette with deionized water.

11) Place A and B beakers into the drying oven and dry at 100-130°C for at least 24 hours.

12) After pans of sand and beakers have dried completely, remove from oven and allow them to cool to room temperature and equilibrate with the humidity in the atmosphere (one to two hours).

13) Weigh each beaker to the nearest 0.001 g.

14) Weigh one aluminum pan (no screen) to the nearest 0.001 g. This pan will be used to weigh the sand fraction of each sample and can be used repeatedly without re-weighing each time. *NOTE: Be sure to get all of the sand out in between weighing.*

15) Weigh each pan + screen + sand + rubble to the nearest 0.001 g for each sample. Dump pan contents into a 250 µm geological sieve, making sure to get all the sand out of the pan and off the screen. Sieve the sample. Pour the sand into the pre-weighed aluminum pan from Step 14; weigh to the nearest 0.001 g.

Notes

1) (Weight of silt/clay in the A beaker) – 0.02 (weight of disperseant/20 mL) x 50 = total weigh of silt/clay (call this F) in the total sample. Percent silt = 100 x (F - weight of clay) / (S + F).

2) (Weight of clay in the B beaker) – 0.02 (weight of dispersant) x 50 = total clay in sample. Percent clay = 100 x weight of clay / (S + F).

3) The percent of sand in the sample is 100S / (S + F), where S is the sand fraction.

Reference

Folk, R.L. 1961. Petrology of Sedimentary Rocks. Hemphills Press. Austin, Texas. 154 p.

Seagrass Percent Cover

Developed by: Kenneth Dunton, Kimberly Jackson and Christopher Wilson
Last updated: December 2009
Approved by: TPWD (2010)

Field Procedures

Three different techniques can be employed to assess vegetative percent cover. The first is the Braun-Blanquet method, which can be applied to both seagrasses and benthic macroalgae. In this method, a diver examines each quadrat underwater. All seagrass species occurring in the quadrat are listed, and a score based on the cover of the species in that quadrat is assigned (Table 1). Cover is defined as the fraction of the total quadrat area that is obscured by a particular species when viewed from directly above. A second more quantitative measure employs 0.25 m^2 quadrats subdivided into 100 5 x 5 cm cells to estimate percent cover of each seagrass species and bare area along a transect. Leaf Area Index (LAI) is a third measure to assess vegetative cover. It is calculated as the product of blade width measurement, shoot length, and shoot density. These data are retrieved from the analysis of seagrass tissue collected in core samples.

Table 1. Braun-Blanquet abundance scores (S). Each seagrass species will be scored in each quadrat according to this scale.

S	**Interpretation**
0	Species absent from quadrat
0.1	Species represented by a solitary short shoot, <5% cover
0.5	Species represented by few (<5) short shoots, <5% cover
1	Species represented by many (>5) short shoots, <5% cover
2	Species represented by many (>5) short shoots, 5-25% cover
3	Species represented by many (>5) short shoots, 25-50% cover
4	Species represented by many (>5) short shoots, 50-75% cover
5	Species represented by many (>5) short shoots, 75-100% cover

Seagrass and Macroalgal Biomass

Developed by: Kenneth Dunton, Kimberly Jackson and Christopher Wilson
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Approved by: TPWD (2010)

Field Procedures

Seagrass Biomass

Three replicate cores are used for estimates of above- and below-ground biomass. A 15 cm inner diameter (ID) core is used to sample *Thalassia*, and a 9 cm ID core is used to sample *Halodule*, *Syrindogium*, *Ruppia* and *Halophila*. Seagrass species composition is determined by visual *in situ* analysis of plants observed within a 25 m radius of each site. A PVC (polyvinyl chloride) core is used for the collection of below-ground and above-ground material. Care is taken to keep only the shoots that reside within the diameter of the core.

Following placement of the 15 cm core on the seabed, the rubber stopper is removed from the top of the core. For both 9 cm and 15 cm cores, before pressing the core into the sediment, the diver runs their fingers carefully around the bottom of the core. If grass has been pulled under the core, it is removed. The diver then presses and twists the core down into the sediment (10-15 cm depth). The stopper is re-installed in the 15 cm core, and the core rocked back and forth. The diver then works their hand under the core and removes it from the grass bed, making sure to keep their hand under the bottom of the core in order to prevent loss of sample.

After emptying the core into a sieve, broken shoots are removed, since these are likely exterior shoots that were cut by the core tube. Samples are then placed in pre-labeled Ziploc bags and immediately placed on ice.

Drift Macroalgal Biomass

Drift macroalgal biomass is determined from the collection of all algal material within a predetermined area. Material from each replicate area is placed in sealed plastic bags and then transported to the laboratory on ice.

Laboratory Procedures

Seagrass and Drift Macroalgal Biomass

Aboveground tissue, including leaves, sheath material and floral parts, are separated from all below-ground tissues. Leaves are carefully cleaned of all attached biota by scraping with a wet cloth or razor blade prior to analysis. All shoots are counted and scaled to the core areain order to obtain accurate estimates of shoot density. The below-ground and above-ground tissues are then placed in separate aluminum envelopes for drying. Sample labels include information on site, species, date collected, shoots or R/R, and number of shoots. Any dead plant material is discarded prior to drying. The live tissue (shoots, roots, and rhizomes) is dried to a constant weight in a 60°C oven and weighed to the nearest milligram. The drying process usually takes 3-

5 days. Lastly, the dry weight biomass for above- and below-ground tissues is used to calculate a root:shoot ratio. For benthic macroalgae, samples are first cleaned of debris and non-algal material. Tissue samples are them dried to a constant weight, weighed, and archived.

Algal Epiphyte Abundance

Developed by: Kenneth Dunton, Kimberly Jackson and Christopher Wilson
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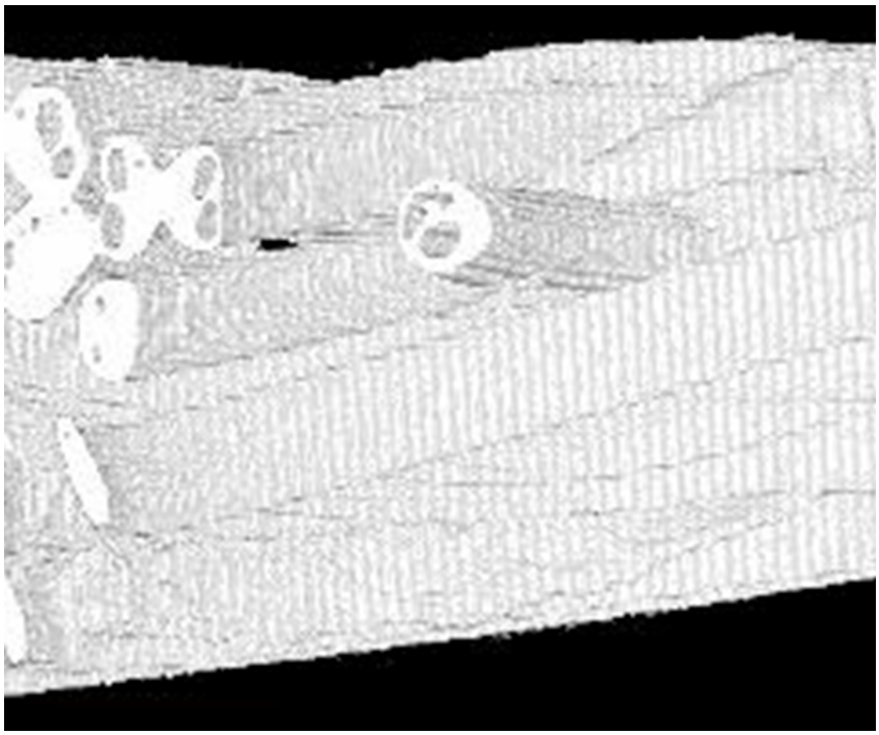
Epiphyte Quantification

Estimates of algal epiphytic biomass are made from separate leaf samples of entire shoots. Leaf samples for epiphytic biomass must be processed within three days of collection. In the laboratory, epiphytes are separated from a known leaf area using a scalpel or razor blade. Scraped material is then collected and retained on pre-weighed glass fiber filters. The collected epiphytic biomass and scraped seagrass leaves are then dried to a constant weight at 60°C for determination of dry weight biomass. Algal epiphytic dry weight biomass is then expressed as both a weight per unit leaf area and as a percent of total dry weight biomass of seagrass tissue. Estimates of epiphyte biomass made on an areal basis are only possible with *Thalassia* and require accurate measurements of the length and width of the area scraped. This is not a valid procedure for the leaves of *Halodule* or *Syringodium* based on the fact that *Halodule* and *Syringodium* leaves are both essentially terete (Fig. 1). In particular, leaves of these species require knowledge of the radius (or diameter) of the leaf for an accurate determination of the surface area, which in turn requires high-resolution three-dimensional (e.g. CT) imagery or microscopy.

Figure 1. CT imagery of *Halodule* leaves illustrating their tube-like morphology (from Christopher Wilson).



Seagrass Tissue Nutrient and Isotopic Analysis

Developed by: Kenneth Dunton, Kimberly Jackson, Christopher Wilson, Karen Bishop and Sang Rul Park

Last Revised: December 2009
Approved by: EPA (2002) and TPWD (2010)

Tissue Preparation

Newly formed leaves (the youngest leaf in a shoot bundle) are gently scraped and rinsed in tap water to remove algal and faunal epiphytes. The rinsed tissue samples are then dried to a constant weight at 60°C and homogenized by grinding to a fine powder using a mortar and pestle.

Tissue C:N Content, δC^{13} and δN^{15}

Tissue samples are analyzed for carbon and nitrogen concentrations and isotopic values using either a PDZ Europa ANCA-GSL elemental analyzer coupled to a PDZ Europa 20-20 isotope ratio mass spectrometer (UC-Davis; precision 0.2 ‰ for 13C and 0.3 ‰ for 15N), or a Carlo Erba 2500 elemental analyzer coupled to a Finnigan MAT DELTAplus isotope ratio mass spectrometer 23 (UTMSI; precision 0.3 ‰ for both $_{13}C$ and $_{15}N$).

Tissue Phosphorous Content

Required Reagents

A. Ammonium Heptamolybdate-Ammonium Vanadate in Nitric Acid

1) Dissolved Dissolve 22.5 g ammonium heptamolybdate [$(NH_4)_6Mo_7O_{24}$. $4H_2O$] in 400 mL DW (a).

2) Dissolve 1.25 g ammonium metavanadate (NH_4VO_3) in 300 mL hot DW (b).

3) Add (b) to (a) in a 1 L volumetric flask, and let the mixture cool to room temperature.

4) Slowly add 250 mL concentrated nitric acid (HNO_3) to the mixture, cool the solution to room temperature, and bring to 1 L volume with DW.

B. Acid Mixture for Tissue Digestion

1) Dilute 165.6 mL concentrated hydrochloric acid (HCl, 37%, sp.gr.1.19) in DW, mix well, let cool, and bring to 1 L volume with DW (2N HCl).

C. Standard Stock Solution

1) Dry approximately 2.5 g potassium dihydrogen phosphate (KH_2PO_4) in an oven at 105°C for 1 hour, cool in desiccator, and store in a tightly stoppered bottle.

2) Dissolve 0.1361 g dried potassium dihydrogen phosphate in DW, and bring to 1 L volume with DW. This solution contains 1,000 µM (Stock solution).

3) Dilute 10 mL Stock Solution to 100 mL final volume by adding DW. This solution contains 100 µM (Stock solution).

Table 1. Volume ratios for standard curve.

Concentration (µM)	**DW (mL)**	**KH_2PO_4 (mL)**	**Stock solution**
0 (Blank)	1.500	0.000	100 µM
25	1.125	0.375	
50	0.750	0.750	
75	0.375	1.125	
100	0.000	1.500	
250	1.125	0.375	1,000 µM
500	0.750	0.750	

Prior to conducting a standard curve analysis:

1) Add 1.5 mL AVM.

2) After 30 minutes, read the absorbance of samples at 410 nm wavelength **(use a blank with ammonium-vanadomolybdate reagent – 1.5 mL DW + 1.5 mL AVM)**.

Procedure

This procedure is based on Chapman and Pratt (1961).

1) Weigh 0.010 - 0.015 g portions of ground plant material in 30-50 mL porcelain crucibles or Pyrex glass beakers.

2) Place porcelain crucibles into a cool muffle furnace, and increase temperature gradually to 550°C. Continue ashing for 5 hours after attaining 550°C.

3) Shut off the muffle furnace and open the door cautiously for rapid cooling. When cool, carefully remove the porcelain crucibles.

4) Dissolve the cooled ash in 1 mL portions 2 N hydrochloric acid (HCl) and mix with a plastic rod.

5) After 15-20 minutes, add 4 mL DW.

6) Mix thoroughly, allow to stand for approximately 30 minutes, and use the supernatant or filter through Whatman No. 42 filter paper, discarding the first portions of the filtrates (optional).

Measurements

1) Pipette 1.5 mL of the digest filtrate or aliquot of the dissolved ash (depending on the procedure used) into a 15 mL glass tube, then add 1.5 mL ammonium-vanadomolybdate reagent.

2) After 30 minutes, read the absorbance of samples at 410 nm wavelength **(use a blank with ammonium-vanadomolybdate reagent – 1.5 mL DW + 1.5 mL AVM)**.

3) Prepare a calibration curve for standards, plotting absorbance against the respective P concentrations

4) Read P concentrations in the unknown samples from the calibration curve.

Calculations

Total phosphorus in plant (%P) =

C (µmol/L) x (1 L/1,000 mL) x 5 mL x (30.9738 µg/µmol) x (1 g/1,000,000 µg) x (1/g sample)

when 5 mL = total volume of the digest/aliquot **(In this method, total volume is 5 mL (1 mL of 2 N HCl + 4 mL of DW))**, and g sample = weight of dry plant used (g).

Reference

Chapman, H.D. and Pratt, P.F. 1961. Methods of Analysis for Soils, Plants and Water. Univ. California, Berkeley, CA, USA.