Environmental Monitoring and Assessment Program
National Coastal Assessment Field Operations

West Coast Field Sampling Methods
Intertidal 2002

Version: 8 August 2002

Amendment to: Environmental Monitoring and Assessment Program
National Coastal Assessment Quality Assurance Project Plan
2001-2004

by

Janet O. Lamberson
Western Ecology Division
Newport, Oregon 97365

Walter G. Nelson, Project Officer
Western Ecology Division
Newport, Oregon 97365

U.S. Environmental Protection Agency
Office of Research and Development
National Health and Environmental Effects Research Laboratory
Western Ecology Division, Coastal Effects Branch
Newport, Oregon 97365

Environmental Monitoring and Assessment Program
National Coastal Assessment
Field Operations

West Coast Field Sampling Methods
Intertidal 2002

Management Approval:
Signature indicates that this QAPP is approved and will be implemented in conducting the research of this project.

Project Leader: Walter G. Nelson — *Signature* [signed] — *Date* 8/7/02

Quality Assurance:
Signature indicates that this QAPP meets the quality requirements of WED.

QA Manager: Craig Mc Farlane — *Signature* [signed] — *Date* 24 Jul 2002

CONTENTS

SECTION . . . . PAGE

1 INTRODUCTION 1

2 OVERVIEW OF FIELD SAMPLING ACTIVITIES - INTERTIDAL 2002 1
Field Activities, Data Forms 1
Site/Sample Identity Codes 2
Field Collection of Environmental Data 3
Site Location 4
Site Description - Station Occupation 6
Sample Data 6

3 INTERTIDAL FIELD SAMPLING METHODS 7
Site Description 7
Site Photos and Habitat 8
Burrowing Shrimp Hole Count and Species Identification 9
Vegetation Cover 10
Composited Surficial Sediment 12
Sediment Temperature and Salinity Measurements 16
Benthic Infaunal Community 16

References 17

Appendix A - Gear List: 18

Appendix B - Sample Data Sheets 19

Appendix C - TOTAL NITROGEN & CARBON BY COMBUSTION METHOD 23

Appendix D - QUANTITATIVE ANALYSIS: PHOSPHORUS, SULFUR, POTASSIUM, CALCIUM, MAGNESIUM, SODIUM, BORON, ZINC, MANGANESE, IRON AND COPPER IN SOILS 25

TABLE 1. Sample handling and storage guidelines for EMAP Coastal 2002 Monitoring. 4
TABLE 2. Sediment Firmness Categories 14
TABLE 3. Chemicals to be measured in sediments by EMAP-Coastal 2002 Monitoring. 15

Figure 1. String gridlines for 0.25 m^2 quadrat 8
Figure 2. Plan for sampling a 1 m^2 quadrat using a 0.25 m^2 quadrat frame. 11

SECTION 1
INTRODUCTION

This document supplements the *National Coastal Assessment: Field Operations Manual* (U.S. EPA, 2001a) and will be used for EMAP West Coast intertidal sampling in 2002 in Washington, Oregon and California.

Procedures for intertidal field sampling in 2002 shall follow those described in the *National Coastal Assessment Quality Assurance Project Plan 2001-2004* (U.S. EPA, 2001b) wherever applicable. The following procedures are based on the 2001-2004 QA Plan and modifications to that Plan are made here as appropriate for 2002 intertidal sampling. Other sources of procedures include the Estuarine Habitat Assessment Protocol (*Puget Sound Estuary Program*), EPA Western Ecology Division protocols, and published methods (Orth and Moore, 1983).

The following parameters will NOT be measured in 2002:

Sediment Toxicity
Water - TSS, chl *a*, nutrients, transmittance, secchi depth, pH, DO
Fish community and tissue samples

SECTION 2
OVERVIEW OF FIELD SAMPLING ACTIVITIES - INTERTIDAL 2002

Field Activities, Data Forms

Intertidal sites will be occupied as appropriate either by boat, hovercraft or on foot. When sites are occupied by boat or overlying water is present, limited water quality samples (salinity, temperature, water depth) will be taken. When sites are occupied at low tide and no water is present, no water quality samples will be possible, but sediment parameters (salinity, temperature) will be measured. It is preferable to occupy stations at low tide when sites are exposed to facilitate burrow counts, plant community, sediment chemistry and benthic community sampling.

Field crews will rely primarily upon hardcopy field data forms to record most field collected data. A generic set of standardized hardcopy forms will be developed for use in each State. Individual states will be allowed to modify the format to accommodate their differences in equipment and to include any additional information or parameters that the state may elect to sample; however, the core Coastal 2002 (C2002) field indicators/data will be recorded in an approved, uniform manner. It is preferred that raw data be recorded with a soft-leaded pencil on rain-protected paper. There should be a separate form for each measurement type; examples of field data sheet types to be used in Coastal 2002 include (see Appendix 2):

Station Information, Composite Sediment Data (p. 23)
Benthic Sample Data (p. 24)
Plant Cover Data (p. 25)

All field sheets must be identified with station ID code and dated; upon completion of the

field entries, the person recording the data will sign each sheet. Field sheets are designed to lead the sampling team through a logical sequence of steps and checks that further ensures sampling protocols are followed. The Field Lead will verify that all field sheets are accounted for and complete prior to departing the sampling station.

Data transcribed from field data sheets to computer files will be verified for accuracy according to procedures outlined in Appendix A of the *National Coastal Assessment Quality Assurance Project Plan 2001-2004* (U.S. EPA, 2001b) which states that each laboratory must establish a system for detecting and eliminating transcription and/or calculation errors prior to reporting data. Suggested procedures for eliminating error include the method where two people work together, one reading from either the original or the copy and the other person following the other version. A second method utilizes a routine available in most software, where the data is re-entered and the program accepts identical entries and rejects unique ones.

Site/Sample Identity Codes

The Regional IM Coordinator will provide each state team with a list of unique site and sample identity (ID) codes. The site codes will be configured in a series specific to each state to include a state's two character abbreviation, year designator, and a sequential numerical series; for example, Washington's sites for year 2002 will be coded **WA02-0001** through **WA02-1000** (or however many sites are designated). Sample ID codes will simply be an abbreviated code to describing the sample type; for example, the sample for sediment organics would be **SO.** Together, the two IDs, **WA02-0001-SO**, constitute a code unique to that sample (Washington, year 2002, site 1, sediment organic chemistry sample). The combined version facilitates the option of barcoded labels, as some regions have so indicated an interest; all the necessary information is on one label.

All core data recorded on field data sheets will be transcribed into the field computer system within a reasonable time following collection (target period, within a week). To ensure consistency, it is preferable that one person be responsible for the data entry. Data entry will be straightforward and user friendly; the fields in the electronic format will closely resemble the hardcopy raw data forms. The hardcopy data forms filled out for a given station will be compiled into a "station data package" and xeroxed to provide in-house working copies for use by the state as well as the copies required by EPA (study files). The original field sheets should be archived by the sponsoring state agency (e.g., Oregon DEQ), as well as, backup disks for all electronic files; the state will retain these raw data on file for at least a 7-year period. The electronic field data file for a station will be transferred to the state IM Coordinator for initial validation and formatting review prior to being transmitted to the centralized Regional Information Management Node (IM Node) where additional validation screening and QC checks will be performed before the data are finally forwarded to the EMAP IM Center at EPA-AED.

Field Collection of Environmental Data

Field sampling will be performed independently by each state; cooperating federal agencies may augment the states' field efforts, both in terms of equipment and personnel. Field crew members will be personnel selected primarily from the respective state environmental agencies. In most instances, 3- to 4-person field crews will conduct the sampling from small craft (typically, 20-25 ft), hovercraft or on foot during a seasonal window spanning from July to mid-September. Sampling is planned as a one-time event per station (i.e., no scheduled repeat sampling for the base sites). However, it is likely that the states, either on their own or in conjunction with other agencies, will continue some elements of the environmental monitoring in following years.

Field activities performed at each site should require approximately 2 hours per site, therefore, a team can expect to sample two to four stations in a normal day; of course, this is subject to such factors as weather, seas, and travel distance between sites. At each sampling site, all C2002 crews will uniformly collect a core set of data and samples following EMAP methods and protocols.

Core field data/samples include (these will be discussed in greater detail in following sections):

- instantaneous water column data (salinity, temperature, depth) - if water column is present
- habitat (general habitat-type and anthropogenic debris or perturbation)
  - digital photographs of the site and notes on shoreline development
- presence of vegetation, burrowing shrimp and other fauna.
- plant community (percent cover, species, biomass and reference specimens)
- sediment consistency and composition, salinity and temperature
- composited surficial sediment, top 2-3 cm, (chemical contaminants - organics and trace metals; total organic carbon, nitrogen, phosphate and grain size)
- benthic macroinvertebrate community structure (composition, abundance, nonindigenous species)

Each state field crew has the option of gathering additional environmental information, as long as those activities are not given precedence over the core activities. Samples collected from the field may be temporarily held at the field staging centers, under appropriate conditions for 1-5 days, to await shipment (or delivered) to centralized storage facilities or processing laboratories. Sample handling and storage guidelines are presented in **Table 1.**

TABLE 1. Sample handling and storage guidelines for EMAP Coastal 2002 Monitoring.

SAMPLE TYPE	CONTAINER	FIELD HOLDING	LAB STORAGE	MAX HOLDING
SEDIMENT:				
Organic contaminants	500 cc cleaned glass jars or I-Chem	Wet ice (4˚C)	Freezer (-20˚C)	1 year
Inorganic contaminants	200-cc Nalgene wide mouth jar	Wet ice (4˚C)	Freezer (-20˚C)	1 year
Total organic carbon, nitrogen, phosphorus	50 cc glass jar	Wet ice (4˚C)	Freezer (-20˚C)	1 year
Silt/clay	125 cc polypropylene wide mouth jar	Wet ice (4˚C)	Refrigerator (4˚C)	1 year
BIOTA:				
Benthos - (1.0 and 0.5 mm sieved within 4 hours)	500-1000 cc wide-mouth Nalgene	10% buffered formalin	Transfer to 70% ethanol	Indefinitely
Shrimp reference specimens	125 cc polypropylene wide mouth jar	10% buffered formalin	Transfer to 70% ethanol	Indefinitely
Plant biomass and reference specimens	Individuals in white plastic trash bags	Wet ice (4˚C)	Herbarium, dry - humidity controlled	Indefinitely

Site Location

The randomly selected sampling locations for each state (or specific study area) will be provided to the field crews as coordinates of latitude/longitude in degrees-minutes, expressed to the nearest 0.01 minute (i.e., 00˚ 00.00'). The crew will locate the sites by use of Global Positioning Satellite System (GPS), preferably, differential (DGPS). Most GPS units display the distance from an entered waypoint as 0.00 nautical miles (nm), therefore this is a convenient unit to use for noting distance from the given coordinates. The acceptable tolerance goal for siting is that the sampling station be established within 0.02 nautical miles (nm), which is equivalent to a radius of approximately 40 m, of the given coordinates. This reflects the accuracy expected from a properly functioning GPS unit of the caliber that will be used for the study. Note: the lat/lon coordinates of the actual collection site or anchorage, not the "intended or given" coordinates,

will be recorded on the field sheet as the sampling location. The GPS's performance should be verified on a daily basis.

Field crews will strictly adhere to the above guidelines for siting the station, unless there are substantiated reasons that prevent sampling within that defined area. Because EMAP's probabilistic sampling design is unbiased, potentially, some of the generated sites can fall in locations that are not amenable to sampling (e.g., inaccessible, on shore, subtidal, rocky bottom, etc.). Preliminary planning by the field team can help resolve these potential problems before they are encountered on the actual day of sampling. Coordinates of the random locations are made available to the teams several months in advance of the field monitoring in order that they have adequate opportunity to formulate logistical plans. The reasonable first step is to plot the given sites on NOAA nautical charts to ascertain the spatial distribution of the sites, then reconnoiter (on paper) the charted locations for obvious problem situations (e.g., water depth, hazards to navigation, etc.). If suspect sites are encountered in this exercise, it is suggested that a field reconnaissance be conducted well ahead of the scheduled sampling to determine actual conditions at the site.

Field teams, however, will have a limited degree of onsite flexibility to relocate sampling sites when confronted with unexpected obstacles or impediments associated with locating within the ±0.02 nm guideline. The crew chief may, for good reason (e.g., outside the sampling frame, danger or risk to crew, excessive rocky bottom, currents, man-made obstructions), move the station to a nearby location from the intended site that is amenable to conduct the sampling. To relocate a site, the field crew will randomly select a direction to move that will take the crew to a suitable location (using a random number table to select a compass heading or a watch with a sweep second hand). The field crew will then walk or navigate in that direction to the edge of the suitable habitat. The distance to the new location within the suitable habitat should then be randomly selected (using a random number table) to bring the field crew to the sampling site, but the new site should be no further than 0.05 nm (100 m), preferably 0.02 nm (40 m), from the original designated site. Using random direction and distance to relocate the site insures that selection of the relocated site is not biased.

When it is necessary to relocate the site more than 0.02 nm, the reason for shift must be documented in the field record. Any site relocation that exceeds 0.05 nm (100 m) will be flagged and reviewed before any data collected from the station are acceptable for inclusion to the study database. The availability of many supplemental sampling site locations for the C2002 design permits the use of alternative sites if a site is found to be unsuitable. If an alternative site is used, it should be the next site on the priority list, *even if it is in a different estuary from the site that is abandoned.*

Site Description - Station Occupation

The following data will be recorded at each station (details to follow):

A. Station number, GPS location, nav type (lu NavType)
B. Date, time, samplers initials, agency (lu01)
C. Sample type, vessel name or walk-in
D. Station failure reasons (lu09)
E. Habitat type (lu41)
F. Shoreline development (lu under development)
G. Weather (lu08), wind speed, direction (luDirection), sea state (luSeaState)
H. Photo - roll and neg #
I. Sediment - composition (lu06), consistency, color (lu26), odor (lu07)
J. Salinity, water temperature, air temperature
K. Burrow count - sp.
L. Burrowing shrimp species (lu shrimp)
M. % Plant cover and biomass
   1. Algae - green, brown, red
   2. *Zostera* - sp.,
   3. Rooted vegetation - sedges, grasses, *Spartina*, etc.
N. Other cover
   1. Clams, crabs, worms (e.g. *Abarenicola*), shell
   2. Debris - specify (lu14)

Several observations will be made in the field to document certain attributes or conditions that will help to characterize the overall ecological health of the site. Observations will be made and noted for the occurrence of macroalgae beds/mats, submerged aquatic vegetation (SAV), or emergent vegetation, the presence of burrowing shrimp, and the occurrence of marine debris. Also, if there is obvious evidence of disruptive anthropogenic activities (e.g., dredging or landfill activity), these observations should be noted with a brief description on the appropriate field form.

Sample Data

Each sample will be assigned a unique sample code number and the following data will be collected for the sample (recorded on data sheet):

A. Station ID, replicate number
B. Sample time, water depth
C. Sample type
D. Sample gear - grab, posthole, other
E. Sample depth, penetration, if applicable
F. Comments

If the station is not flooded and is occupied on foot or by hovercraft or airboat, sediment and benthic community samples will be taken by hand as described in Section 3. If the station is covered by water and occupied by boat, sediment and benthic community samples will be taken by grab using methodology similar to that used in 1999-2000 and described in the *National Coastal Assessment Coastal 2000 Quality Assurance Project Plan 2000-2004*.

SECTION 3
INTERTIDAL FIELD SAMPLING METHODS

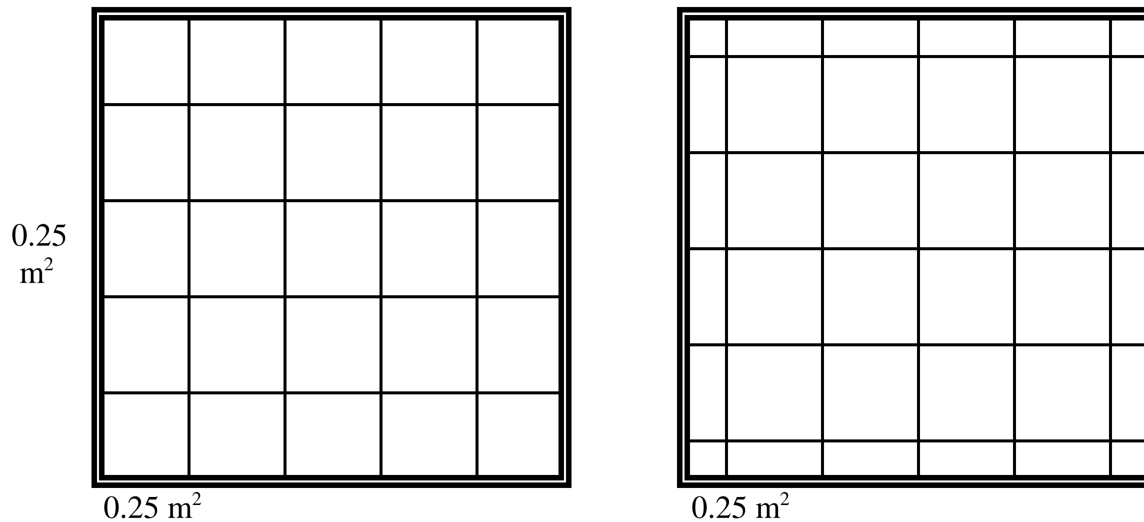
Site Description

The sample frame for the 2002 intertidal samples is 0.0 tide (mean lower low water) to annual mean high water (the calculated average of all observed daily lower and higher high tides), which may differ between estuaries and at different locations within an estuary. This intertidal area extends into lower salt marsh areas and is often demarcated at the upper edge by a wrack line of drift plant material and light buoyant materials. Large woody debris is deposited at the higher high water line, at the upper edge of the salt marsh, which is above the 2002 defined sample frame. Sample sites within the 2002 sample frame will be flooded with sea water at tide levels equivalent to the calculated mean high water. . . . .

Upon arrival at the site, the station number, GPS location and water depth, date, time, samplers' initials and agency (lu list 01), will be recorded on the data sheet. For GPS location, type of GPS unit (GPS, DGPS) will be indicated on the data sheet - DGPS is preferred. Vessel name (or walk-in) will be recorded, and if the station is abandoned, the reason for abandonment will be recorded on the data sheet (lu 09). Data will be recorded on sea state (lu Sea State), weather, wind speed and direction, and estimated tidal level (taken from predicted tide charts) as well as air temperature and habitat type (lu 41).

A 0.25-m^2 quadrat will be randomly placed on the sediment at the GPS-located site by the following method: a member of the sampling crew will look briefly at a water resistant watch with a sweep second hand held horizontally to the sediment surface to determine which way the second hand is pointing, and will turn to face in that direction. The quadrat will be dropped behind the crewperson over the shoulder to eliminate bias in placing the quadrat. If the quadrat lands in an area that already has footprints from the approach of the sampling crew, the quadrat will be moved to the nearest spot beyond the footprints. The 0.25-m^2 quadrat will be turned over three times as described below to define a 1-m square sampling site (see Fig. 2). If a digital watch is used, the seconds indicator can be used to determine which direction to face to place the quadrat, by turning in the direction that a sweep second hand, if present, would point. A random number table may be used to select the direction the quadrat is thrown, for example a table of numbers from 1 to 360 to indicate degrees of a circle with north at 0 degrees. The purpose is to eliminate sampler bias in the initial placement of the quadrat. The quadrat should have gridlines made of string to divide the area into uniform squares - a useful design is four strings in each direction, forming 25 equal squares. Another design is five strings in each direction forming 16 equal squares, with half squares around the edges, and quarter squares in the corners, providing 25 string intersects. Either design is useful for burrow counts and estimating plant cover (see Fig. 1). . . . .

Figure 1. String gridlines for 0.25 m^2 quadrat



Site Photos and Habitat

Three to five photos and notes will be taken to document site characteristics and anthropogenic impact such as shoreline construction, dredging or recreational use. A digital camera would be useful for this documentation, because the images could be downloaded to electronic storage. The first of a series of photos from a site should be of the quadrat in place and should include a site and date label. It is advisable to make the label in large black letters on a tan or gray photo neutral density background to eliminate glare and overexposure of the label in the photo. The datasheet should record information to identify additional photos, such as a description of the image. Photos will be taken of a) the quadrat in place, b) the general area of the sampling site including the quadrat, and c) the nearest shoreline (1-3 photos of the shoreline), as well as d) any unusual nearby impacts or activities such as dredging, oyster culture, or the presence of any large trash or structures. If possible, the camera should be set to record date and time of the photograph. A description of the site will be recorded on the datasheet, with reference to shoreline development as well as observed impacts such as oil sheen or debris found at the site.

Habitat is defined by the presence or absence of factors such as dominant plant or animal species that impact the abundance and number of species in the benthic infaunal community. If rooted plants such as *Zostera* spp., *Spartina* or marsh plants are present, the habitat is defined as the dominant plant genus present. If no plants are present, but there are numerous (i.e. >5 per 0.25 square meter) burrow openings, the habitat is defined as the genus of burrowing shrimp, *Neotrypaea* or *Upogebia* (or other burrowing species) present. The presence of oysters or abundant shell debris defines the site as oyster or shell habitat. If no rooted plants, burrowing shrimp, shellfish culture etc. are present, the habitat is defined by its geological type - rocky, gravel, coarse or fine sand, muddy sand, sandy mud or mud. If unattached drift macroalgae are present, the habitat will be defined as if there were no drift algae there, but the presence and abundance of the algae will be recorded on the data sheet. The following habitats may be encountered: Tidal Flat Sand, Tidal Flat Mud, Port or Marina, Rocky Bottom (Gravel or Rocks and Boulders), Burrowing Shrimp (*Neotrypaea*, *Upogebia*), SAV *(Zostera marina*, *Z. japonica)*,

Shellfish Bed, Shell Deposit Area, Salt Marsh (*Spartina*, Sedge Marsh, etc.) - see lu list 41.

Burrowing Shrimp Hole Count and Species Identification

A wide variety of invertebrates construct burrows in marine and estuarine sediments. Of special interest to EMAP are those constructed by burrowing shrimp (*Neotrypaea* spp. and *Upogebia spp.*), which are U-shaped with lateral tunnels that typically have two or more burrow openings at the sediment surface (i.e., burrow holes). Shrimp burrow holes range in diameter from ~1 mm to ~30 mm; for burrow hole counts, only holes >3 mm (=1/8") diameter will be counted because smaller holes may belong to other invertebrate species. Burrows constructed by *Neotrypaea* are not mucus lined (i.e., to the finger, the inside of the burrow wall feels like soft, granular sediment) and have small volcano-shaped mounds, often (but not always) with ex-current channels extending downwards from the "summit" burrow opening. Burrows constructed by *Upogebia* have a mucus lining (i.e., to the finger, one can feel a slick surface and firmly-packed sediment forming the burrow wall), the burrow openings are often larger in diameter than those of *Neotrypaea*, and the burrow openings usually are not associated with pronounced mounds. At *Neotrypaea* sites, deposits of shrimp feces are often noted. At some sites, both *Upogebia* and *Neotrypaea* may be present. If the identity of shrimp at the site is unknown, reference samples should be collected with a shrimp gun and either examined for identification, or saved (on ice or preserved in formalin) for later identification. The number of burrow holes will be counted in one 0.25-m^2 square quadrat at each sampling site.

Comparability - This procedure below will be used for all samples when counting burrow holes. One person will count burrow holes in the quadrat (see procedure below), taking care to disturb the quadrat as little as possible. Data will be recorded in the appropriate space on the data sheet. The quadrat will remain in place. Another person (or persons) will count the burrow holes in the quadrat, taking care to disturb the plot as little as possible. The quadrat will remain in place. Burrow hole count measurements will be compared. If the counts differ by more than 10%, the sampling personnel will discuss why the counts may have differed, a re-count of burrows will be performed by all persons involved until the counts are less 10% apart.

Procedure - If vegetation or debris is present within the quadrat, it will not be moved. The sediment surface will not be disturbed (which could fill in burrow holes). It may be helpful to use a hand tally counter. Using the grid lines as a guide, all of the burrow holes >3 mm diameter *within the bare (i.e., not covered by plant material) areas only* inside the quadrat will be counted. A rule-of-thumb will be established for counting holes that lie under grid lines to ensure that those holes are not double-counted. For example, only holes that lie under the bottom or left lines of each grid cell will be counted, and not holes that lie under the top or right lines of a grid cell. The number of holes per 0.25-m^2 quadrat will be recorded on the data sheet. If vegetation covers ≥50% of the quadrat (i.e., bare area cover is <50%), the density (#/area) of burrow holes under the vegetation will be estimated. To do this, the vegetation will be gently pulled back taking care to disturb the sediment surface as little as possible, and the density of burrow holes under the vegetation will be visually estimated to be equal to, less than, or greater than the density of holes on the bare substrate. This information will be recorded on the data sheet in the comments section.

Vegetation Cover

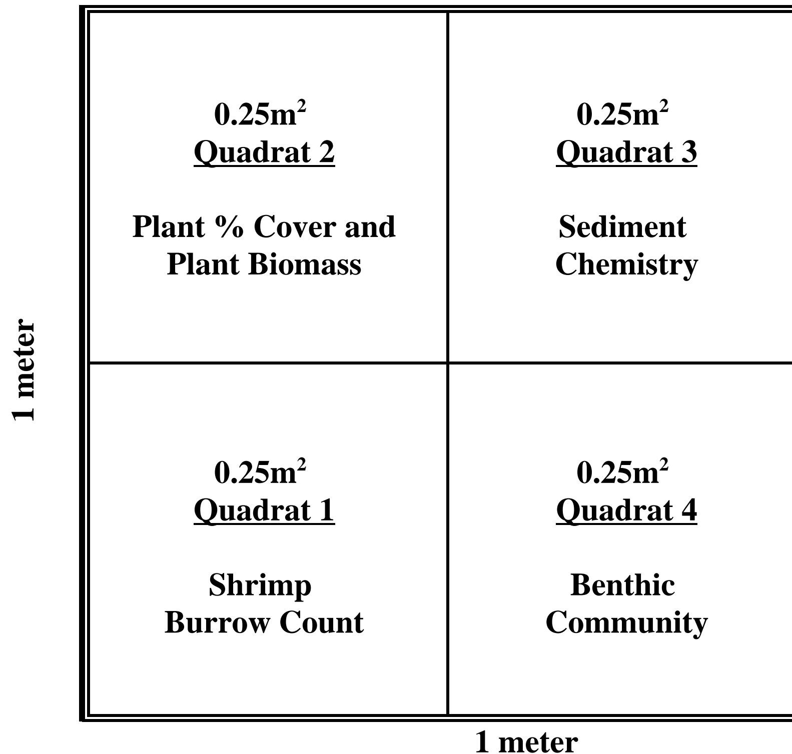
Where rooted plants (e.g. seagrasses, marsh plants, *Spartina*) or drift algae are present, the plant community will be characterized by taking quantitative measurements of the percent cover of the vegetation, using a 0.25 m^2 quadrat, a 100-m measuring tape marked at random locations, and an estimate of percent plant cover in the benthic community sample. Details of these procedures are presented below. Plant transects and percent cover need not be done if no vegetation is present, but the absence of vegetation must be noted on field sheets.

Rooted plant species such as seagrasses will be recorded separately from algae, which may be recorded only to the "taxonomic" level of red, green and brown algae. Seagrasses and other rooted plants will be identified to species if possible, or to the lowest practical taxonomic level. If the species of plant is not known with certainty by the field crew, a reference specimen will be taken by the field crew, placed in a white plastic trash bag with a label inside the bag identifying the sample site and a reference number for the plant that can be referred to when the plant is identified by a plant taxonomist. The reference number will be used to record the presence of the plant at the site on the datasheet, then the reference number will be replaced with the plant name when available. The bag containing the reference sample will be held on ice until the plant can be preserved in an herbarium plant press for identification by a qualified plant taxonomist.

Percent Cover and Plant Biomass in a 0.25 m^2 Quadrat -

Determination of percent cover in a 0.25-m^2 quadrat will be done after the random placement of the quadrat and counting of burrow holes. After counting burrow holes in one 0.25-m^2 quadrat area, the quadrat frame will be turned over in a direction away from the sampler to form the second quarter of the total 1 m^2 sampling area (see Fig. 2).

Figure 2. Plan for sampling a 1 m^2 quadrat using a 0.25 m^2 quadrat frame.



Percent cover within the 0.25-m^2 quadrat will be visually estimated separately for green, brown or red macroalgae, *Zostera* spp., *Spartina* or other rooted plant genera present, and bare (i.e., open, unvegetated) substrate. A sampling plot is defined by the 0.25 m^2 quadrat. Plant species cover is the percentage of a sampling plot that is overlain by the canopy of each type of plant occurring in the plot and of any bare substrate. Cover for bare substrate is the percentage of a sampling plot that is not overlain by any plant. The maximum cover for any individual species of plant or bare substrate is 100%. The maximum total cover possible for all species of plants in a plot is 100% times the number of species of plants in the plot, and thus may be greater than 100% if several species overlap at different layers of cover.

Comparability - The procedure below will be used for all samples. People sampling will discuss how they each arrived at their estimates as a way of determining what corrective measures should be taken. Care will be taken to disturb the plot as little as possible. Data will be recorded in the appropriate space on the data sheet. The quadrat will remain in place.

Procedure - The percent of the plot occupied by bare substrate will be estimated and recorded on the data sheet. The percent of the plot overlain by each type of plant (i.e., *Zostera*, green algae, brown algae, red algae) will be estimated. Upper layers will be gently lifted to see

what is underneath to determine how much of the plot is actually occupied by each type of plant being estimated. Estimates will be recorded on the data sheet. It may be helpful to use a visual guide for estimating percent cover (e.g., Orth and Moore, 1983), or percentage of plant cover at the intersection of grid lines strung into the quadrat frame may be used.

For each rooted species, the maximum plant height (height above the soil or sediment surface for upright or creeping forms) or blade length of the longest blades (recumbent forms such as *Zostera* spp.) will be recorded on the plant cover data sheet. No shoot counts will be made. The total biomass of each species of rooted plants and each type of algae will be determined by cutting all vegetation at the sediment surface, sorting by species, and obtaining the dry weight (g dry weight) of biomass for each species. For drift green algae, if the total algal cover is equal to 100%, the algal biomass sample may consist of 25% of the 0.25-m^2 quadrat, but the area sampled must be recorded on the datasheet. Plants may be semi-dried in the field. Grasses and sedges may be air dried, while algae, succulent or large-leaved plants may be dried using an herbarium press or food dryer. Alternatively, plants may be frozen in the field for drying at the laboratory. Plants will be dried in the laboratory until they have reached constant dry weight, generally requiring several hours or overnight at 80°C.

Plant Transect Line -

Determination of plant cover (if present) at 25 randomly located points along a 5-m transect will be done by pre-marking a 100-m transect tape with 25 randomly located points within each of 20 5-meter lengths along the tape. A random number table will be used to randomly select one of the 20 5-meter lengths. The 5-m section of the tape that is selected will then be laid out oriented parallel to the shoreline with its center (the 2.5-m point of that 5-m section) at the center of the 1-m^2 sample site (see Fig. 2). At each of the 25 random points along the 5-m transect, each plant species present at that point (or open ground) will be recorded with an "x" on the percent plant cover data sheet (p. 26). More than one plant species may be noted at each point if plants overlap at different layers of cover. The total number of "x's" (number of times a species is encountered at the 25 random points) will be recorded at the bottom of the page for each species. Multiplying the total number of "x's" per species times 4 will give percent cover along the transect for that species: $(n/25) \times 100 = n \times 4 = n\%$.

In place of the 100-m tape, a 5-m transect stick or tape marked at 1-cm intervals may be used, with 25 random points (25 of the 500 cm points) selected from a random number table. Percent plant cover of each plant species will also be noted in the benthic core taken later, and this estimate will also be recorded on the plant cover data sheet.

Composited Surficial Sediment

At each site, sediment from the surficial sediment layer (top 2-3 cm) will be collected by spatula or scoop and composited to provide sufficient sediment (~1 liter) for the analyses of inorganic and organic chemical contaminants, total organic carbon (TOC), total nitrogen and total phosphorus, and grain size determinations. The quadrat frame that was used in shrimp burrow counts and plant cover will be turned over to the right to complete the third quarter of the 1-meter square sample plot (Fig. 2). The quadrat will be pressed slightly into the sediment to demarcate

the sample area, and then removed to facilitate sampling the surficial sediment layer. Notes will be recorded on the data sheet describing sediment composition and consistency (see Table 2), color and odor. Presence of drift items, debris and shell, etc. within the general area (5 m diameter circle) will be recorded on the data sheet. Sediment will be removed with clean scoops, spoons and other sampling devices only and will not be touched with bare hands. Root material should be excluded. As stated in Appendix A of the *National Coastal Assessment Coastal 2000 Quality Assurance Project Plan 2000-2004* (U.S. EPA. 2001b), field personnel must strictly adhere to EMAP protocols to ensure the collection of representative, uncontaminated sediment chemistry samples. These sample collection protocols are described in detail in the *National Coastal Assessment: Field Operations Manual* (U.S. EPA. 2001a). If insufficient surface sediment can be collected from within the 0.25 m^2 square allocated for this purpose, additional sediment may be taken from other areas, preferably within the one meter square quadrat sampling area, as long as the sediment has not been disturbed by handling. Sediment chemical samples will be analyzed for contaminants listed in Table 3, using protocols and QA/QC procedures outlined in the *National Coastal Assessment Coastal 2000 Quality Assurance Project Plan 2000-2004* (U.S. EPA. 2001b).

Surficial sediment from the area within the quadrat will be composited in the field in a clean, high-grade stainless steel or Teflon vessel prior to distribution to the various sample containers. Each addition of sediment to the composite will be blended in by stirring and the final mixture will be stirred well to ensure a homogenous sample before sub-samples for the various analyses (Table 3) are taken as follows:

Organic chemical contaminants - approximately 500 cc of composited sediment will be placed in a clean, prelabeled, glass wide-mouth, 1-pint Mason jar or I-Chem jars (see B5 for QC requirements). The sample will be held on wet ice until transfer to shore storage, the sample should then be frozen unless it is scheduled for extraction within 7 days; in that case, the sample may be held at 4˚C to await processing.

Inorganic chemical contaminants - approximately 200 cc of composited sediment will be placed in a clean, prelabeled, wide-mouth Nalgene jar. The sample will be held on wet ice until transfer to shore storage, the sample should then be frozen unless it is scheduled for digestion within 7 days; in that case, the sample may be held at 4˚C to await processing.

Total organic carbon, total nitrogen, total phosphorus - approximately 30 cc of composited sediment will be placed in a small, clean, prelabeled glass bottle/jar. The sample will be held on wet ice until transfer to shore storage, the sample should then be frozen to await further laboratory analysis. Appendices C and D give methodology for analysis of total nitrogen and phosphorus in sediments.

Grain size determination - approximately 120 cc of composited sediment will be placed in a clean, prelabeled, wide-mouth polypropylene jar. The sample will be held on wet ice and, upon transfer to the shore storage, the sample will be held at 4˚C (sample is not to be frozen) to await further laboratory processing.

TABLE 2. Sediment Firmness Categories

Sediment Firmness Category	**Substrate Lithology**	**Personal Effect on Substrate**	**Push Test (rod/pencil)**
Very Soft	Very soft clays (with slurry surface) No plant roots – no additional support to the sediment matrix	Immediate/severe sinkage into sediment	No pressure required to fully insert a rod/pencil
Soft/Loose	Very soft to soft clays (with coherent surface) Loose/soft silty sands Minimal vegetation/roots – not supplying much additional support to the sediment matrix	Some support generated by the sediment, but sinkage occurs to some degree Sinkage worsens unless keep moving around	Minimal pressure required to fully insert a rod/pencil
Firm/ Medium Dense	Soft to firm clays Medium dense sand / mixed granular sediment Some cobbles/pebbles/shells Some vegetation/roots – supplying some additional support to the sediment matrix	Sediment supports body weight, but there is some impact on the substrate surface (Clay holds footprint shape / sand shifts when walked on)	Fair degree of pressure required to fully insert a rod/pencil. Some wiggling of rod/pencil required, but does insert fully
Hard/ Very Dense	Firm to stiff clays Dense, compacted sands High spatial density of cobbles/small boulders/shells High concentration of vegetation/ roots – supplying large degree of additional support to the sediment matrix	Surface totally supports body weight, with minimal/no impact on substrate surface (stationary or walking)	Large degree of pressure / wiggling required to insert a rod/pencil Or - will not insert fully

TABLE 3. Chemicals to be measured in sediments by EMAP-Coastal 2002 Monitoring.

Polynuclear Aromatic Hydrocarbons (PAHs)

Acenaphthene
Anthracene
Benz(a)anthracene
Benzo(a)pyrene
Biphenyl
Chrysene
Dibenz(a,h)anthracene
Dibenzothiophene
2,6-dimethylnaphthalene
Fluoranthene
Fluorene
2-methylnaphthalene
1-methylnaphthalene
1-methylphenanthrene
2,6-dimethylnaphtalene
Naphthalene
Pyrene
Benzo(b)fluoranthene
Acenaphthylene
Benzo(k)fluoranthene
Benzo(g,h,i)perylene
Indeno(1,2,3-c,d)pyrene
2,3,5-trimethylnaphthalene

21 PCB Congeners

PCB No.	Compound Name
8	2,4'-dichlorobiphenyl
18	2,2',5-trichlorobiphenyl
28	2,4,4'-trichlorobiphenyl
44	2,2',3,5'-tetrachlorobiphenyl
52	2,2',5,5'-tetrachlorobiphenyl
66	2,3',4,4'-tetrachlorobiphenyl
101	2,2',4,5,5'-pentachlorobiphenyl
105	2,3,3',4,4'-pentachlorobiphenyl
110/77	2,3,3',4',6-pentachlorobiphenyl 3,3',4,4'-tetrachlorobiphenyl
118	2,3',4,4',5-pentachlorobiphenyl
126	3,3',4,4',5-pentachlorobiphenyl
128	2,2',3,3',4,4'-hexachlorobiphenyl
138	2,2',3,4,4',5'-hexachlorobiphenyl
153	2,2',4,4',5,5'-hexachlorobiphenyl
170	2,2',3,3',4,4',5-heptachlorobiphenyl
180	2,2',3,4,4',5,5'-heptachlorobiphenyl
187	2,2',3,4',5,5',6-heptachlorobiphenyl
195	2,2',3,3',4,4',5,6-octachlorobiphenyl
206	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl
209	2,2'3,3',4,4',5,5',6,6 '-decachlorobiphenyl

DDT and its metabolites

2,4'-DDD
4,4'-DDD
2,4'-DDE
4,4'-DDE
2,4'-DDT
4,4'-DDT

Chlorinated pesticides other than DDT

Aldrin
Alpha-Chlordane
Dieldrin
Endosulfan I
Endosulfan II
Endosulfan sulfate
Endrin
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
Lindane (gamma-BHC)
Mirex
Toxaphene
Trans-Nonachlor

Trace Elements

Aluminum
Antimony (sediment, only)
Arsenic
Cadmium
Chromium
Copper
Iron
Lead
Manganese (sediment, only)
Mercury
Nickel
Selenium
Silver
Tin
Zinc

Other Measurements

Total organic carbon, total nitrogen and total phosphorus in sediments

Sediment Temperature and Salinity Measurements

Sediment temperature and salinity will be taken in the fourth quadrat square as the benthic core (described below) is taken. Sediment temperature will be taken by inserting a field thermometer 5 cm into the sediment. Salinity will be taken by measuring the salinity of the water that accumulates at the surface of the sediment as surficial sediment is taken or as the benthic community core sample is taken, using a refractometer, or a salinity sample may be taken and held in a well-sealed vial to be analyzed later. Thermometers and refractometers should be calibrated daily by comparison to a standard thermometer (temperature) and distilled water or reference saline solution (salinity).

Benthic Infaunal Community

Benthic infaunal samples will be collected using a post-hole corer to take a 0.1-m2 sample of the benthic community down to a depth of 10 cm. The use of alternative gear must be documented with a full description of both the gear and techniques. The sample will be taken in the fourth quarter of the 1-m^2 sample area by replacing the 0.25-m^2 quadrat frame in the area used for sampling the composited sediment, then turning the frame over toward the sampler so that the fourth quarter of the 1-m square is completed (Fig. 2). The benthic sampler will be placed within this 0.25-m^2 area (after the quadrat frame is removed), and pushed down to a depth of 10 cm. Stones, oyster shell, etc. that are mostly in the sampler should be included in the sample, while those that are mostly outside of the sampler should be removed to facilitate pushing the sampler down into the sediment. Estimate plant cover within the benthic sample and record this information on the plant cover datasheet.. If marine plant material that lies flat on the sediment is present (e.g., *Zostera*, algae) it should be included in the sample. Blades and roots should be cut at the edge of the sampler with a knife or sharpened shovel so that only plant material within the sample area is included in the sample. If *Spartina*, sedges or other standing plants are present, the shoots, stems and leaf blades may be cut off just above the sediment surface, but the root material should be included in the sample down to a depth of 10 cm. Root material below that depth should be cut off with a sharpened shovel as the sample is taken. Sediment with plant and other included material will be scooped into a large container for transport to the sieving station.

The total volume of the benthic sample is 10 liters. If a post-hole corer is used, four core samples will be taken within the 0.25-m^2 quadrat area to collect the benthic sample. The calculated (from EMAP 1999 data) average depth penetration of the 0.10-m^2 benthic grab used in previous EMAP collections is 10 cm, equivalent to the target depth penetration with the post-hole sampler. The sampling area of the post-hole sampler is .025 m^2, so four samples with the post-hole sampler will be equivalent in area and volume to one 0.10 m^2 benthic grab sample.

The collected sediment will be held on ice in a cooler until processed (within 4 hours) or immediately processed aboard a boat or ashore by sieving the entire contents of the grab through a 1.0-mm standard sieve. All organisms and detritus retained on the screen will be gently transferred to labeled, wide-mouth, Nalgene containers and preserved with buffered formalin (7-10% final concentration). Details of field procedures for sieving, preserving and processing benthic samples as well as for labeling sample containers are outlined in the *National Coastal Assessment: Field Operations Manual* (U.S. EPA. 2001a).

The formalin preserved samples will be forwarded to a benthic ecology laboratory for sorting, identification, and counting of organisms. At the laboratory, it is recommended that the formalin-fixed samples be transferred to 70% ethanol within 2 weeks of field collection to avoid undue deterioration of sample integrity that may further complicate identification (e.g., loss of heads/appendages and erosion of shells or exoskeletons).

References

Orth, Robert J. and Kenneth A. Moore. 1983. Submersed vascular plants: Techniques for analyzing their distribution and abundance. *Marine Technology Society Journal* 7:38-52.

U.S. EPA. 2001a. National Coastal Assessment: Field Operations Manual. United States Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. EPA 620/R-01/003. pp72.

U.S. EPA. 2001b. Environmental Monitoring and Assessment Program (EMAP): National Coastal Assessment Quality Assurance Project Plan 2001-2004. United States Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. EPA/620/R-01/002. pp198.

Appendix A - Gear List:

Toboggan to carry gear
Waders, boots, mudders, life jacket if in boat or hovercraft
Radio, other safety equipment - in backpack
GPS or DGPS
Ice chest for holding samples, ice
Data sheets, pens, pencils
Grab or posthole sampler for benthic samples
0.25 m^2 quadrat for vegetation and burrow count, with string gridlines
Scoops, spoons, shovel, garden trowel
Small spoons for filling sample jars
Watch with sweep second hand, Compass
Waterproof camera - digital preferred
Labels for photo ID
Refractometers and pipettes or salinity vials
Thermometers
Metric ruler to measure grab penetration depth
Metric tape to measure plant height
Reel tape or survey rod for plant transect
Hand tally counter for shrimp burrow counts
Sample containers for composited sediment samples -
    TOC, chemistry, grain size, % moisture
Sample containers for benthic samples (bucket, jars)
White plastic trash bags for plant reference specimens, bags for shrimp specimens
Serrated breadknife and sharpened garden spade for plant and benthic samples
Sieves - 1.0-mm, tubs to sieve in
Formalin, buffered
Stain - Rose Bengal
Other - towels, gloves, label tape, waterproof label paper, scissors, markers, buckets, funnels, vinyl tape for sealing jars, duct tape, field logbooks, forceps to pick screen, squirt bottle, beige or gray 3X5 cards, tide tables or tide chart
Herbarium press, paper
Shrimp gun
Lab keys

Float plan or foot plan
Collecting permit, estuary map

Appendix B - Sample Data Sheets

A - Station Information, Composite Sediment Data
B - Plant, Sediment and Benthic Community SampleData
C - Plant Cover Data

EMAP 2002 Station Number ________________________
Date (e.g. 24/Aug/2002) ____________________________
Time ____________________________
Samplers Initials ____________________________
Agency ____________________________
GPS Actual Station Location (circle one: GPS DGPS) -
Lat ___________________ **Long** _____________________
GPS Verification:____
Vessel Name or Walk-in ____________________________
Water depth (m) ____________________________
Estimated tide level (m) ____________________________
Station Failure Reason - if station is abandoned ___________________________________
Habitat Type (Lu41) ____________________________
Shoreline development ____
Weather (circle) Clear Hazy Part cloudy Cloudy Fog Drizzle Rain Thunderstorm
Wind Speed/Direction ____________________________
Sea State ____________________________
Air Temperature ____________________________
<u>Take 3-5 Photos</u> - Roll # _______________________ **Neg #** __________________

Sediment Composition (circle below)
mud sandy mud muddy sand fine sand coarse sand gravel cobbles
Color ____________________________
Odor ____________________________
Sediment firmness (circle - see Table 2)
Very Soft Soft/Loose Firm/Medium Dense Hard/Very Dense

Burrow Count per 0.25 m^2 ____________________________
Species of Shrimp ____________________________
<u>Collect reference specimens of burrowing shrimp if in doubt</u>

Other (check if present):
Clams _______ **Crabs** _______**Worms (e.g. *Abarenicola*)** _______ **Shell** __________
Debris - (specify) ____
Drift Plants ____
<u>Comments:</u>
Recorder_____________________

EMAP 2002 Station Number ____________________

<u>SAMPLE PLANT COMMUNITY -</u>

see plant cover data sheet for plant height (blade length), percent cover and species (with reference number), other plants observed

<u>SAMPLE COMPOSITED SEDIMENT</u>

Time ________________________

Water depth ________________________

Grab or surface sample? ________________________

Number of grabs ________________________

Grab penetration (cm) ________________________

Sample only top 2 cm of sediment. Stir and subsample for inorganic chemistry (200 cc), organic chemistry (500 cc), TOC (30 cc), grain size (125 cc)

<u>SAMPLE BENTHIC COMMUNITY</u>

Time ________________________

Water depth ________________________

Grab or surface sample? ________________________

Number of grabs ________________________

Percent plant cover in core __________________

Plant species ____

Sediment temperature ________________________

Salinity ________________________

Grab penetration (cm) ________________________

<u>Comments:</u>

Recorder______________________

Replace this blank page with EXCEL spreadsheet

“Percent Plant Cover datasheet - Page 22"

Appendix C - TOTAL NITROGEN & CARBON BY COMBUSTION METHOD
Soil Method

Summary -
This analytical method quantitatively determines the total amount of nitrogen and carbon in all forms in soil, botanical, and miscellaneous materials using a dynamic flash combustion system coupled with a gas chromatographic (gc) separation system and a thermal conductivity detection (tcd) system. The analytical method is based on the complete and instantaneous oxidation of the sample by "flash combustion" which converts all organic and inorganic substances into combustion gases (n_2, no_x, co_2, and h_20). The instrument has a detection limit of 0.01% and is generally reproducible within " 5.0%.

Sample Preparation -
- Samples should be spread out and air-dried where the sample will not be contaminated. Samples may also be placed in a forced draft oven at 55ºC.
- Large chunks of sample are broken up with a rubber hammer and samples are crushed in a Pico-Braun soil pulverizer to pass through a 2 mm sieve.
- A subsample is pulverized to pass through a 60 mesh sieve to ensure homeogeneity, placed in an appropriate container and labeled.

Equipment and Apparatus -
- Nitrogen/Carbon Gas Analyzer, Carlo Erba 1500, series 2 with combustion furnace, gas chromatographic separation system and thermal conductivity detection system.
- Electronic microbalance: 2000 mg capacity.
- Tin capsules, 5x9 mm (Costech 041061).

Reagents -

• Combustion:	Chromium (III) Oxide (Costech 011001)
	Silvered Cobalt (II,III) Oxide (Costech 011007)
• Reduction:	Copper wires, reduced (Costech 011012)
• Water Trap:	Magnesium Perchlorate (Costech 021022)
	Quartz chips (Costech 021025)
	Molecular sieves 3A, 1/16" (Costech 021023)
• Calibration Standard:	Atropine (4.84%N; 70.56%C) (Costech 031042)

(CAUTION: Atropine can be hazardous. Read label before use and follow all precautions. See MSDS).

Procedure -
- Sample preparation is the most critical part of this analysis. To ensure that you are providing accurate, reliable data, a finely ground homogeneous mixture that could pass through a 50-mesh sieve is necessary when using 10 mg to 20 mg sample sizes that are weighed into a 5x9 mm tin capsule.
- Using an electronic microbalance weigh out about 20 mg of soil sample into a tared tin capsule, encapsulate and record sample weight to the nearest 0.001 mg.
- Following the SIOP for the Carlo Erba, initialize and calibrate the instrument. Enter sample

weights and analyze unknown samples for total nitrogen and total carbon. Report results to the nearest 0.01%.

Standards and Spiking -
- Atropine (4.84%N; 70.56% C) is used to calibrate the instrument.

Instrument Set-up -
- Initialize the instrument following manufacturers suggested protocol.
- Conduct a system leak check on the combustion system.
- Perform blank stabilization test by analyzing consecutive blanks until the blanks stabilize at a constant value.
- Standardize the instrument using Atropine.

Quality Control and Reference Material -
- Duplicate subsamples should be weighed out, digested and analyzed for the first, last and every tenth sample of each set.
- The standard reference material for this analysis is Gridley (0.097 ± 0.02 % N and 0.886 ± 0.12 % C). This standard should be analyzed every ten samples.

Calculations -

MDL for this analysis is 0.01 % N and 0.01 % C.

Health and Safety -
- Atropine can be hazardous. Read label before use and follow all precautions.
- The Copper wires used as a reductant and the Magnesium Perchlorate are hazardous and after use must be disposed of according to U. C. Davis E. H. & S. policies.

Special Handling and/or Cleaning Procedures -
- Due to the potential for contamination of samples, tweezers must be used to enclose the sample in the tin capsule and to place samples into autosampler.

Comments / Notes -
- Tin foil capsules are utilized as a combustion catalyst.
- All soil calibration samples should be checked for homogeneity and verified using standard addition techniques and a chemical standards such as EDTA or sulfanilic acid.

Literature and References -

Dumas, J. B. 1981. Sur les procédés de l'analyse organique. Annal. de Chimie, XLVII. p. 195-213.

Pella, E. 1990. Elemental organic analysis: part 1: historical developments. American Laboratory. Feb. 22:116+.

Pella, E. 1990. Elemental organic analysis: part 2: state of the art. American Laboratory. Aug. 22:28+.

Appendix D - QUANTITATIVE ANALYSIS: PHOSPHORUS, SULFUR, POTASSIUM, CALCIUM, MAGNESIUM, SODIUM, BORON, ZINC, MANGANESE, IRON AND COPPER IN SOILS

Soil Method

Summary -
This method quantitatively determines the concentration of Zn, Mn, Fe, Cu, Mo and a variety of other elements in soil samples utilizing a nitric acid/hydrogen peroxide microwave digestion for preparation with subsequent analyte concentration determined by atomic absorption spectrometry (AAS) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The methodology utilized a pressure digestion/dissolution of the botanical materials and is incomplete relative to the total oxidation of organic carbon. K, Na, Zn, Cu, Mn, and Fe are analyzed by AAS and all others are analyzed by ICP-AES with vacuum spectrometer. The method has a detection limit of approximately 0.01% for P, Ca, and Mg and 0.1 mg kg^{-1} for the others. The method is generally reproducible within " 8.0% for all analytes.

Sample Preparation -
- Samples should be spread out and air-dried where the samples will not be contaminated. Samples may also be placed in a forced draft oven at 55ºC.
- Large chunks of sample are broken up with a rubber hammer and samples are crushed in a Bico-Braun soil pulverizer to pass through a 2 mm sieve.
- Samples are transferred to plastic soil cups with airtight lids and labeled appropriately.

Equipment and Apparatus -
- Analytical balance: 250 g capacity, resolution " 0.1 mg.
- Microwave digestion system and teflon PFA double wall digestion vessels (equipped with 200 psi relief seals, CEM PN-221003, Box 200 Matthews NC 28106).
- Repipette dispensers, calibrated to 0.5 " 0.05 mL and 2.0 " 0.8 mL.
- Polypropylene centrifuge tube with cap, 15 mL graduated.
- 16x100 mm autosampler tube.
- Atomic Absorption Spectrophotometer (AAS).
- Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), Thermo Jarrell Ash IRIS.

Reagents -
- Deionized water, ASTM Type II grade.
- Micro® clean detergent (Baxter Sci. PN - C6286-6).
- Nitric Acid, Trace Metal Grade, 12 *N*.
- Hydrogen Peroxide 30% solution.
- Standard Calibration solutions of P, S, K, Ca, Mg, Na, B, Zn, Mn, Cu, and Fe. Prepare four multi-element standards: of K, Ca, Mg ranging from 5-500 mg L^{-1}; P, S, and Na ranging from 1.0-100 mg L^{-1}; and B, Zn, Fe, Mn and Cu ranging from 0.10-10.0 mg L^{-1}. Dilute standard calibration solutions with deionized water and 2% nitric acid by volume.
- Standard calibration solutions for other elements should be prepared from 1000 mg L^{-1} stock solutions in concentrations of 0.0, 5.0 and 10.0 mg L^{-1}.

Procedure -

- Weigh 500 " 5.0 mg of dry botanical material (see comments) and place in 120 mL teflon digestion vessel (dilution factor 30:1).
- Using repipettes add 0.5 mL of trace metal grade concentrated nitric acid and 2.00 ml of 30% Hydrogen Peroxide to each vessel (see comments). Ensure that the sample is completely wetted by the reagents.
- Place digestion vessel in outer body shell, cap and allow the plant sample and reagents to predigest for 10 minutes. Close vessel (see comments) and release valves and place samples (twelve vessels) in the microwave and set microwave program for 5:00 minutes of 296 watts power (40% power) and 8:00 minutes of 565 watts power (90% power).
- At completion remove samples and place in hood to cool (optional - place in freezer to cool for 20 minutes). Vent vessels by rotating release valve 2 revolution. Remove cap, rinse cap into vessel with deionized water.
- Cap centrifuge tube, label, invert three times and place in storage rack (see comments).
- Determine P, S, B, Ca, and Mg at 185.9, 182.0, 208.9, 315.8, and 279.0 nm wavelengths, respectively by ICP-AES (see comments). Determine Zn (213.7 nm, slit 0.7), Mn (279.5 nm, slit 0.2), Fe (248.3 nm, slit 0.2) and Cu (324.8 nm, slit 0.7) respectively by AAS. Determine Na (589.0 nm, slit 0.7) and K (766.4 nm, slit 0.7) by AES. Record results as mg L^{-1} of analyte to three significant digits.

Standards and Spiking -

- The ICP-AES should be calibrated using a four-point calibration. The standards are 0.0, 20.0, 200.0 and 400.0 mg L^{-1} Ca and 0.0, 10.0, 100.0 and 200.0 mg L^{-1} Mg.
- The standards should be prepared in 500 mL volumetric flasks using the UCD-3A as follows:

Standard Name	18 megaohm water (mL)	HNO_3 (mL)	UCD-3A (mL)
STD1	490	10.0	-
STD2	485	10.0	5.0
STD3	440	10.0	50.0
STD4	390	10.0	100.0

- The AA should be calibrated using a seven-point calibration. The standards are 0.0, 0.1, 0.2, 0.5, 1.0, 2.0 and 3.0 mg L^{-1} of Zn and 0.0, 0.25, 0.5, 1.0, 2.0, 3.0 and 5.0 mg L^{-1} of Cu, Mn and Fe.
- Prepare a 100 mg L^{-1} standard of Zn and another 100 mg L^{-1} standard of Cu, Fe, and Mn by diluting 10 mL of a 1000 mg L^{-1} standard to 100 mL with 18 megaohm water using a volumetric flask. The combination standard of Cu, Fe and Mn will include 10 mL of each 1000 mg L^{-1} standard. These 100 mg L^{-1} standards will be used to prepare the calibration standards.
- The standards should be prepared in 100 mL volumetric flasks using 100 mg L^{-1} standard solutions as follows:

Standard Name	H20 (mL)	HNO3(mL)	100 mg L^{-1} Zn (mL)	100 mg L^{-1} Cu, Mn, Fe (mL)
STD1	98.0	2.0	0.0	0.0
STD2	97.7	2.0	0.1	0.25

STD3	97.3	2.0	0.2	0.5
STD4	96.5	2.0	0.5	1.0
STD5	95.0	2.0	1.0	2.0
STD6	93.0	2.0	2.0	3.0
STD7	90.0	2.0	3.0	5.0

- A duplicate of the last sample in each set should be weighed out for a spike. Add 500 uL each of UCD3A, Micro6 and 1000 mg L^{-1} each of K and Na to this sample and a reagent blank. (Spike level will be 66.7 mg L^{-1} Ca, 33.3 mg L^{-1} each of Mg and Na, 16.7 mg L^{-1} each of P and S, 1.67 mg L^{-1} of B, 0.166 mg L^{-1} each of Cu, Fe and Mn and 0.10 mg L^{-1} of Zn in solution or 0.40 % Ca, 0.20 % Mg, 0.20 % Na, 0.20 % K, 0.10 % P, 1000 mg kg^{-1} S, 100 mg kg^{-1} B, 10.0 mg kg^{-1} each of Cu, Fe and Mn and 6.0 mg kg^{-1} of Zn on a dry matter basis.)

Instrument Set-up -

- The microwave is preprogrammed for the standard digestion method that includes five (5) minutes at 40% power followed by eight (8) minutes at 90% power. This method will be used for all digestions unless otherwise noted.
- The **ICP-AES** should be turned on following the instrument standard operating procedure and allowed to warm up for 45 minutes.
- After warm up, run a wavelength profile and record the dX and dY coordinates in the log book.
- The following settings should be used for Ca, Mg, P, S and B analyses:

Method:	CMBSP
Power setting:	1150 Watts
Pump setting:	100 rpm
Nebulizer pressure:	0.65 psi
Flush time:	45 sec
# of Repeats:	3

- All data should be printed as well as stored in an appropriately named data file, including the work request number (i.e. P325.txt)
- Upon completion of analysis, export data for transfer to LIMS, rinse nebulizer and spray chamber with 2% HNO_3 and shut down following the instrument standard operating procedure.
- The **AA** should be turned on following the instrument standard operating procedure and allowed to warm up for approximately 10 minutes.
- For Na and K the instrument must be set to Emission mode (EM).
- In the Energy mode, set wavelength to 589 nm for Na or 766.4 nm for K and fine tune using the 1000 mg L^{-1} standard. Once wavelength has been set, adjust gain to approximately 100 while sipping 1000 mg L^{-1} standard.
- Turn on printer and laptop computer.
- Open and name a data file on the laptop.
- Make sure that the print button on the AA is on and then begin analysis.
- For all other analyses the instrument must be set to Absorbance mode (ABS).
- Place the combination cathode lamp in the turret and set lamp current to 25 mA.
- While aspirating the highest calibration standard, optomize the wavelength and burner assembly position.
- Analyze the samples at the following wavelength and slit settings:

  Zn - 213.9 nm, slit 0.7 H

Fe - 248.3 nm, slit 0.2 L
Mn - 279.5nm, slit 0.2 L
Cu - 324.7nm, slit 0.7 H

- Turn on printer and laptop computer.
- Open and name a data file on the laptop (e.g., P216Cu.txt).
- Make sure that the print button on the AA is on and then begin analysis.
- Upon completion of analyses, instrument should be shut down following instrument standard operating procedures.

Quality Control and Reference Material -

- Duplicate samples from each set will be included. Duplicate samples include the first and last sample of every set and every tenth sample in between.
- A reagent blank will be included with each sample set.
- A spike duplicate and blank spike will be included with each sample set.
- Standard reference materials will be included with each sample set. The standard reference materials will include UCD155, UCD107, UCD187, UCD 2891. For sample sets that require Mo, Nist1577b will also be included. For sample sets that require Cd, Cr, Ni and Pb, ASH and SOIL standards will also be included.

Calculations -

For P, Ca and Mg report results to three significant digits on a percentage (%) basis:

For S, B, Na, Zn, Mn, Fe, Cu and all others report results to three significant digits on a mg kg^{-1} basis:

Spike recovery(%) = $(C_{sp} - C_s / C) * 100$

$$mg\,kg^{-1}\,analyte = \frac{(mg\,L^{-1})\,x\,(DF)}{\%DM/100}$$

Dilution Factor (DF) = 60, DF for samples that require Cu is 3(

C_{sp} = Concentration of spiked sample mg L^{-1}
C_s = Concentration of sample mg L^{-1}
C = Concentration of spike mg L^{-1}

MDL = 0.01 % Ca, Mg, and P
MDL = 0.1 mg kg^{-1} for all others

Health and Safety -

- Personal protective equipment, including gloves, goggles and lab coat will be worn when handling acids required for microwave digestion.
- Samples will be allowed to cool to room temperature prior to transfer from digestion vessel to prevent burns.
- All vessel components must be dry and free of particulate matter. Drops of liquid or particles

will absorb microwave energy, causing localized heating which may char and damage vessel components, leading to possible vessel failure.
- ICP-AES waste is considered a hazardous waste and must be disposed of according to U. C. Davis E. H. & S. policies.

Special Handling and/or Cleaning Procedures -
- Upon completion of transfer of digested sample, add 5 mL of Microclean to the PFA vessel and allow to it for approximately 15 minutes.
- Scrub vessel with brush to loosen and remove any undigested material.
- Rinse vessel with ASTM Type II water and place in 0.5 N HCl acid bath to soak for 30 minutes.
- Remove vessel from acid bath, rinse three times with ASTM Type II water and place in oven to dry.

Comments / Notes -
- Check repipette dispensing volume, calibrate using an analytical balance.
- When adding reagent to vessels always wear protective clothing (i.e., eye protection, lab coat, disposable gloves and shoes). Always handle reagents and opening of vessels in an acid hood capable of high air flow, 100 cfm.
- Inspect vessel rupture seal in the cap for replacement.
- Centrifuging may be necessary to clear the digest.
- Samples having analyte concentrations exceeding the highest standard will require dilution and reanalysis.

Literature and References -
Anderson, D. L. and L. J. Henderson. 1986. Sealed chamber digestion for plant nutrient analyses. Agron. J. 78:937-939.

Kalra, Y. P., D. G. Maynard, and F. G. Radford. 1989. Microwave digestion of tree foliage for multi-element analysis. Can. J. For. Res. 19:981-985.

Kingston, H. M. and L. B. Jassie. 1986. Microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. Anal Chem. 58:2534-2541.

Kingston, H. M. and L. B. Jassie. 1988. Monitoring and predicting parameters in microwave dissolution. p. 93-134. In: Kingston and Jassie (ed.) Introduction to microwave sample preparation. American Chemical Society, Washington DC.

Meyer, G. A. and P. N. Keliher. 1992. An overview of analysis by inductively coupled plasma-atomic emission spectrometry. p. 473-505. In: A. Montaser and D.W. Golightly (ed.) Inductively coupled plasmas in analytical atomic spectrometry. VCH Publishers Inc. New York, NY.

Sah, R. N., and R. O. Miller. 1992 Spontaneous reaction for acid dissolution of biological tissues in closed vessels. Anal. Chem. 64:230-233.

Stripp, R. A. and D. C. Bogen. 1989. The rapid decomposition of biological materials by using a microwave acid digestion bomb. J. Anal. Toxicol. 13:57-59.