RPwG
(A)

A. **Title:** **SURVEY OF INJURED TIDAL MARSHES IN PRINCE WILLIAM SOUND AND THE GULF OF ALASKA**

B. **Study ID Number:** 12

C: **Project Leaders:**

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D. **Lead Agency:**

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E. **Cost of Proposed Work:** Phase I: (FY91) $15,000.

F. **Inclusive Dates of Study Plan:** Phase I: June-September, 1991.

G. **Signatures:**

C Callahan

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Survey of Injured Tidal Marshes in the Gulf of Alaska and Prince William Sound

I. Introduction

In March 1989 the *Exxon Valdez* ran aground on Bligh Reef in Prince William Sound, spilling approximately 11 million gallons of Prudhoe Bay Crude Oil into the water and impacting over one thousand miles of coastal resources in the Prince William Sound (PWS) and the Gulf of Alaska (GOA). The *Exxon Valdez* oil spill (EVOS) affected the region's environmental habitat, including both floral and faunal populations, as well as recreational, educational, and aesthetic attributes.

Tidal marshes have been classified as the most sensitive shore-type to oil pollution (Ganning et al., 1984). It has been estimated that 2-20 years are required for tidal marshes to recover naturally (Cairns and Buikema, 1984; RPWG, 1990). Oil is rapidly buried in marshes because they are low energy systems, and degradation is limited under the anaerobic conditions found in these environments (Cairns and Buikema, 1984).

Natural marsh recovery begins when oil toxicity is reduced to a point that can be tolerated by recolonizers (Baker et al., 1990). Full tidal marsh recovery hinges on reduction in oil toxicity; availability of propagules; stability of sediments; and biotic interactions (Getter et al., 1984). Restoration activities in heavily oiled marshes may be expected to require both substantial effort and extended time periods. The presence of oil in high concentrations at a site may complicate restoration efforts, and vegetation regrowth in these areas may occur slowly, if at all.

The coastal areas in the PWS and the GOA consist of varied rocky shores, with many small inlets and coves. A qualitative survey conducted by EPA over several days in August of 1990 indicated that approximately 20% of the marshes visited were impacted. For example, two tidal marshes, the Bay of Isles and Tonsina Bay, were observed to still be heavily impacted by oil and lacking in natural regeneration of oil-affected vegetation. (see attached maps). The EPA qualitative survey (August, 1990) noted (1) heavy effects from residual oil (approximately one acre of mixed Carex and Triglochin) and suspected effects to 1/4 to 1/2 acre of Zostera at the Bay of Isles, and (2) extensive effects to Puccinellia from residual oil at Tonsina Bay; Glaux and other species at higher elevations in this marsh were not apparently affected. See Attachment A for maps of Prince William Sound and the Gulf of Alaska, as well as detailed maps of the Bay of Isles and Tonsina Bay showing oiled areas as based on the oil spill maps.

The Restoration Planning Work Group (RPWG) has recommended that a comprehensive survey is needed to determine which, if any, tidal

marsh sites should be restored. Although tidal marshes seem to represent a relatively small percentage of the coastline affected by EVOS, their actual abundance and relative importance in the coastal ecosystem within the oil spill area has not been established. Generally, in most areas, these types of habitats are ecologically important, serving as feeding and resting areas for migratory waterfowl and other birds and as alternative food sources for browsing mammals in PWS/GOA.

II. Objectives

The primary objective of this study is to assess the relative abundance of tidal marsh habitat within the impacted area of Prince William Sound and Gulf of Alaska; the relative value of this type of habitat in the oil spill area, and the amount of this habitat impacted by EVOS. Phase I constitutes a preliminary survey to be done in 1991 which will include an analysis of existing databases and limited field work. At this time, only Phase I is being considered for implementation in Summer 1991. Based on this preliminary survey, if restoration of marsh habitats appears necessary and is justifiable, a more detailed assessment (Phase II) of sites to determine which specific sites could require restoration may also be done later this year. A feasibility study and actual site restoration activities at certain sites may follow in 1992.

III. Methods

i) Phase I - Preliminary Survey:

Investigators will begin with an analysis of the existing databases to identify a list of all potential marsh habitat in PWS/GOA. The databases were established by the state of Alaska in conjunction with the oil spill response effort and incorporate information gathered from surveys of the oil spill area over the past two years. Investigators will also include and consider the initial list of sites identified in the EPA August 1990 qualitative assessment.[1] Further attempts will be made to verify injured tidal marshes through discussions with local/regional/state personnel, particularly those involved in the Spring 1991 Surveys (MAYSAP). Data forms and field notes from the specific beach segments may be analyzed. Once a preliminary list of sites is identified as a result of these initial reviews, a field survey may be initiated to verify potential sites.

General site assessment information will be collected during this initial field survey effort. A site description will be initiated using the Tidal Marsh Restoration Data Form (see attachment A). These forms should also include any notations (from

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[1] Sites manifesting some impact include: Block Island Fuel Dump, fringe effect in center of Triglochin marsh; Crafton Bay, fringe effects in Carex marsh; Bay of Isles East Arm, fringe effects to Carex marsh; Marsha Bay, fringe effects to Plantago; Elrington East from North Twin Bay, fringe effects to Puccinellia.

the existing oil spill maps and survey results) of the amount of oil originally estimated to be present in the area (i.e., light, moderate, heavy) and this information can be updated in the field. Sites will be evaluated in order to determine their classification in terms of wetland type. Besides vegetative cover, some of parameters noted below (Phase II) and on the Marsh Restoration Data Form should be described, as determined appropriate by scientists onsite. In addition, any preliminary observations relative to habitat functions and value will also be noted. Although a detailed analysis of wetland functions and values will not be done in conjunction with this effort, an attempt will be made to ascertain any existing information relative to the value of these wetland habitats in the oil spill area.

Phase I should provide comprehensive assessment of the amount of wetland habitat type within the oil spill area. This information will be made available to RPWG, along with a recommendation as to whether restoration of marshes within the oil spill area is warranted.

ii) Phase II - Detailed Site Assessment:

Following RPWG review of the preliminary survey, a more detailed assessment may be recommended for some sites. Specific information, described below, will be collected for those sites identified as impacted and possibly needing restoration. The Marsh Restoration Data Form may be completed for those sites having the most potential for restoration. During site assessment, the following will be accomplished:

(1) Care will be taken not to injure sites with equipment or foot traffic. All instruments will be calibrated prior to field use.

(2) Complete Part A (Restoration Assessment) of the Example Tidal Marsh Assessment Data Form (Attachment A).

(3) Observations involving biomass, fringe impacts, percent cover, and vigor will rely on the experience and professional judgement of the investigator.

(4) Dig holes in the substrate at various points with a spade, to determine if roots and rhizomes are present. This will indicate the areal extent of the marsh.

(5) Use a tape measure and measure area impacted to determine if area is greater than 10 m^2.

(6) Based on site assessment results, provide recommendation for restoration. If the decision is not to restore an impacted site and allow natural recovery, note as such and move to the next site. If the decision is not to restore a marsh because it is not impacted, fill out Part B (Donor Site Assessment) of the Example

Tidal Marsh Assessment Data Form (Attachment A). If the answer to questions 3-5 on Part B of the data form are yes, then recommend as a donor site and move to the next site to be investigated.

(7) If the site is to be restored and fertilizer applied, establish a permanent reference point and take soil samples, including duplicates (number to be statistically determined), using a random number table to choose the sample points. Use a 6.5 cm diameter plexiglass piston cover, place the sediment into solvent rinsed foil, wrap, and store (Burns and Teal, 1979). Place labels on each sample and code with a unique I.D. number. Place tape over the label to ensure it adheres to the sample and does not smear. Place samples in a cooler with cooler packs and transport to lab for nutrient/pH analysis. Rinse all utensils with redistilled solvents before use (Burns and Teal, 1979).

(8) Take a picture of the site and mark in a log book the film frame and roll number.

(9) Measure salinity and water temperature at all sites to establish ambient site conditions.

Parameters to be noted include:

- measure impacted area

- take a picture of site

- determine reference point, substrate ID and presence if living/dead rhizomes

- determine extent of living/dead vegetation biomass - Evaluating vegetation below ground in the absence of aboveground cover is especially important, because rhizomes present in the substrate indicate the areal extent of a marsh. The presence of obviously viable or nonviable rhizomes will influence the method of restoration chosen.

- take soil sample, if appropriate (nutrient, pH analysis). The soil samples should be analyzed for organic content, nutrients (total plant-available N,P,K,Ca and Mg), pH, and salinity at the Soil Science Lab at Oregon State University. Total hydrocarbon and weathered hydrocarbon fractions will be analyzed by SAIC Inc. in San Diego, CA. It will be important to relate revegetation success (survival rate) to particular oil fractions present. Tidal marsh species are elevation specific, and this factor may play an important role in establishing a stand for a particular species.

- ambient site conditions (salinity, water temperature, etc.).

It is important to note the flushing characteristics of the site since this will be an important consideration relative to potential eutrophication upon use of fertilization.

If a marsh does not exhibit visible impact from oil (reduced cover or vigor) within the following guidelines, no restoration actions will be recommended.

- apparent cover - This criterion provides information on the extent of the visibly impacted area. The extent of the original marsh will be estimated through historical data, if available, or the presence of living or dead rhizomes in the substrate. If a significant amount of the original marsh area (e.g. approximately 20%) exhibits a large reduction in vegetative cover (e.g. approximately 80%), then the marsh will qualify for restoration. In addition, if the impacted area is greater than 10 m^2, site restoration is also triggered. This accounts for situations in which a small percentage of a large marsh is injured, but the total area affected is rather large. These two criteria are considered the minimum size criteria for restoration activities, but are only guidelines. The final decision for restoring a site will depend on best professional judgement in the field. Information generated under this criterion will serve as the "injured baseline" from which restoration success (assessed through vegetative cover) will be measured.

- vigor - This criterion evaluates visible stress on the ecosystem independent of apparent cover, through judgement of plant health (i.e., brown dying plants versus green healthy plants). Therefore, even if there is a large percent of vegetative cover in an impacted tidal marsh, if a large quantity of it is of low vigor (brown and dying), then the marsh would qualify for restoration. The actual trigger for restoration requires low "vigor" on greater than 20% of the original marsh area.

In addition to potential restoration sites, field observations in the summer of 1990 have identified several potential donor sites (transplant sources for revegetation):

1) Tidal marsh at the head of Outside Bay on Naked Island, (Carex)
2) Tidal marsh on Crafton Island, (Carex)
3) East Bay tidal marsh on Perry Island, (Carex)

4) Tonsina Bay, (Puccinellia)
6) Fringe tidal marsh around the Bay of Isles and Marsh Bay on Knight Island.

Although these sites may have potential as donor sites, they have not been investigated in detail. Therefore, the following information will be collected to evaluate potential donor sites:

- Species present - The composition of a tidal marsh will factor into its potential to serve as a donor site, based on the species requiring replacement at the Bay of Isles and Tonsina Bay. The site must also have an abundant supply of the appropriate species for revegetation of the Bay of Isles and Tonsina Bay.

- Oil impact - A donor site must be an "unstressed" system (void of unnatural perturbations outside of natural stress), and therefore lacking in any apparent impact from oil.

- Historical treatment record - Again, since a donor site must be "unstressed" relative to the Bay of Isles and Tonsina Bay, a potential donor must not have been subjected to any type of treatment or cleanup operations.

- Vigor - To qualify as a donor site, a tidal marsh must exhibit nearly 100% cover of healthy vegetation, again demonstrating the importance of an "unstressed" system.

- Proximity of vegetative donor site - It is cost-effective and ecologically prudent to choose a donor site in close proximity to the Bay of Isles and Tonsina Bay.

- Size of donor plot - The donor site must be large enough that collection of plants for transplantation will not adversely affect the donor. Less than 1 percent cover will be removed from each donor site.

iii) Phase III - Feasibility Study/Additional Information:

Following Phase II, restoration planning may continue and include a feasibility study, and also gathering additional specific information for each potential restoration site. Additional specific information may be needed in order to assess transplanting method, fertilization necessity, and to determine appropriate donor site.

Once it has been determined through site assessment that a site will be restored, information gathered during site assessment will be instrumental in prescribing the appropriate technique for site restoration. The method chosen will also determine the logistical effort required to implement the restoration effort. The

feasibility of transplanting into the substrate at a site must be evaluated prior to choosing transplantation as a restoration method. For example, the presence of shale at Snug Harbor will make transplantation difficult. Transplantation of vegetation will be recommended for all sites where there is a large reduction in vegetative cover (e.g., 80%) over a significant portion of the original marsh area (e.g., 20%).

If a large portion of a marsh (e.g., 20%) has vegetation showing visual symptoms of stress (e.g., brown and dying) but there is a significant amount of vegetation cover present (e.g., more than 20%), fertilizer may be applied to strengthen existing plants and aid in recovery. If fertilization is recommended, the substrate will be sampled for nutrient and oil fraction analyses prior to nutrient application to determine the proper fertilization rate. All other individual tidal marshes will be evaluated on a case-by-case basis, and the appropriate restoration technique chosen based on site-specific conditions. The appropriate donor site will be selected based on information gathered during site assessment and the following criteria:

Proximity of vegetative donor site - It is cost-effective and ecologically prudent to choose a donor site in close proximity to the site to be restored.

Species affected/to be replaced - To be a donor site, the site must have an abundant supply of the appropriate species for revegetation of a disturbed area.

Size of donor plot - The donor site must be large enough that collection of plants for transplantation will not adversely affect the donor. Less than 1 percent cover will be removed from each donor site.

Actual restoration activities may be initiated in 1992. Should revegetation occur at that time, monitoring will occur annually and investigators will observe each fertilized/revegetated site for cover (measure of success). The Tidal Marsh Site Monitoring Form (Attachment B) will be used to monitor the revegetation success at a site. Vegetation may decline in the second and third years after planting, indicating the need for long-term monitoring and possibly additional restoration activities before success can be achieved.

IV. Quality Assurance/Quality Control (QA/QC)

See attached QA/QC document.

V. Logistics

Logistics will be difficult in Alaska due to the remoteness of the location and will most likely include the use of float planes and possibly helicopters.

VI. Duration

The duration of Phase I of this study is approximately three months. Following that, Phase II may be implemented in mid- to late- Summer, 1991. It will be important to accomplish as much of the field work in the 1991 field season as possible, especially if it is determined that a feasibility study and potential restoration are warranted.

VII. Data Analysis

The study involves site assessment including visual observations of vegetative cover. The study will not involve full ecosystem structure and function analyses.

VIII. Schedules and Planning

Following the initial data base search, a planning meeting will be convened by the project officer with the Co-Principal Investigators and any cooperating scientists, if applicable, to prepare detailed plans for both logistical support and field schedules. Logistical support, including purchase and organization of equipment and materials required for field work, scheduling for air flights and boats will be handled in Alaska. Phase I site visits are tentatively planned for early July, 1991.

If warranted, Phase II site visits will be planned for later in the Summer. Preliminary results will be reported to the Oil Spill Restoration Planning Work Group, USEPA ERL-C and Region X.

IX. Budget

Estimated Cost:

Cooperating Scientist (Technicians)	$ 1,000
Travel	2,500
Site (float plane)	5,000
Analysis (computer, lab, mapping)	6,000
Supplies and Equipment	500
TOTAL (Phase I)	$15,000

Funding levels for 1992 and subsequent years contingent on restoration/monitoring need as determined by success of 1991 program.

* Examples of relevant field equipment are as follows:

Field data sheets, clipboards, pencils, spades, measuring tapes, camera, thermometers, salinity meters, 6.5 cm diameter piston corers, solvent rinsed foil, cooler packs, coolers, ziploc bags for soil samples, tape, labels, gloves, rain gear, log book.

** Funding levels for 1992 and subsequent years are contingent on restoration/monitoring need as determined through evaluation of the 1991 program.

X. PERSONNEL QUALIFICATIONS

See attached resumes for professional qualifications.

IX. CITATIONS

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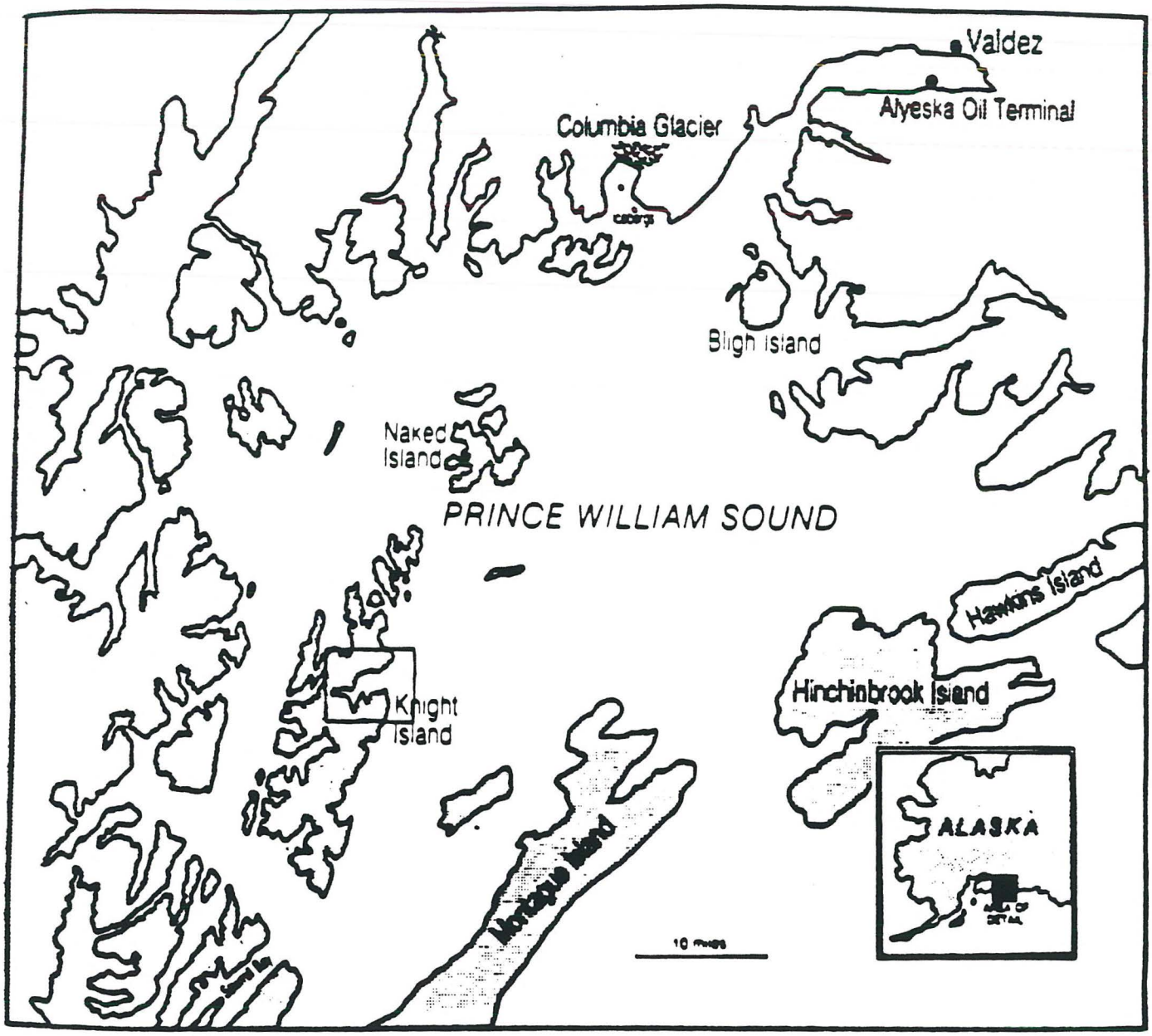
Maps

Prince William Sound

Bay of Isles, Knight Island

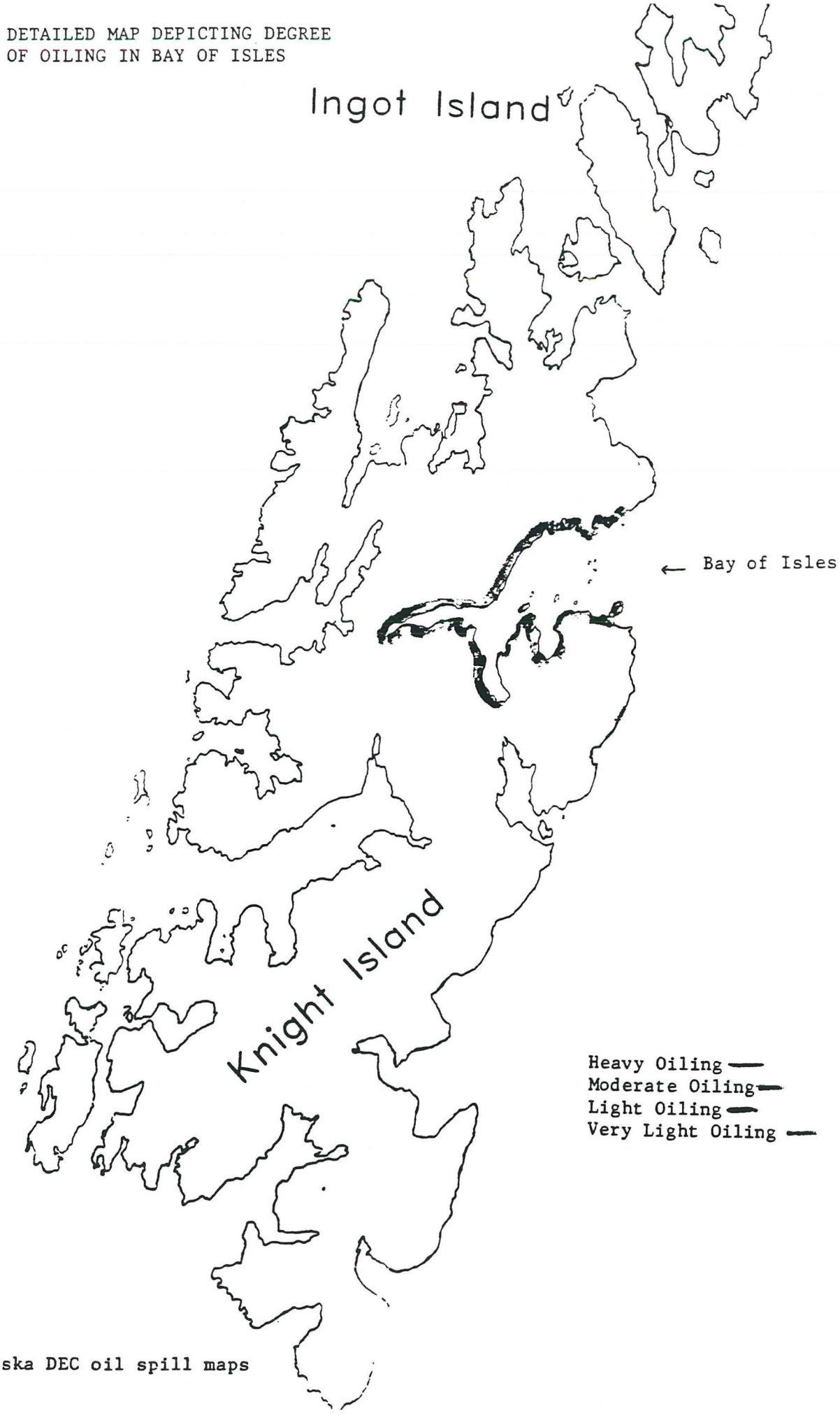
Kenai Peninsula

Tonsina Bay

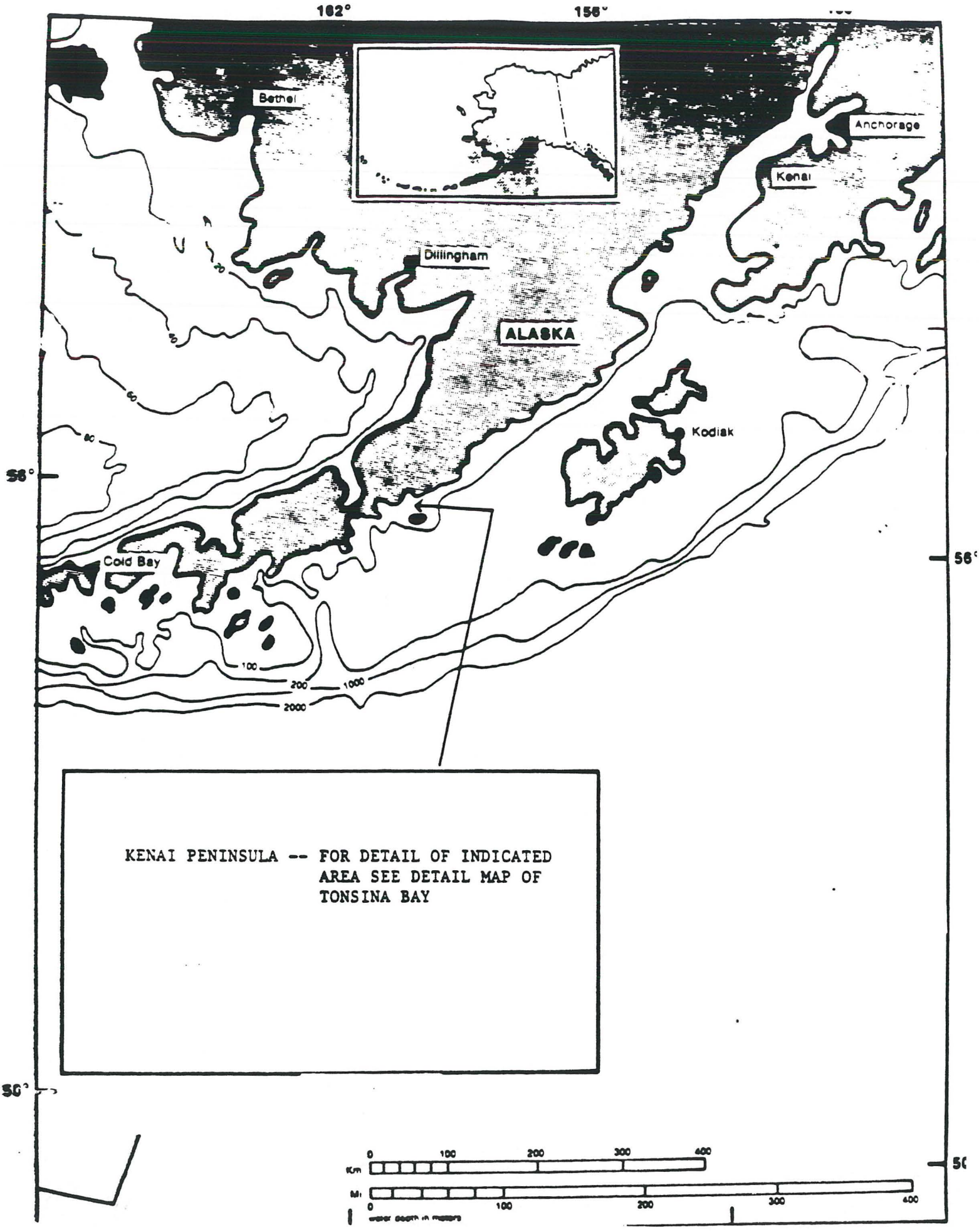


PRINCE WILLIAM SOUND -- FOR DETAIL OF BLOCKED AREA SEE
DETAIL MAP OF BAY OF ISLES

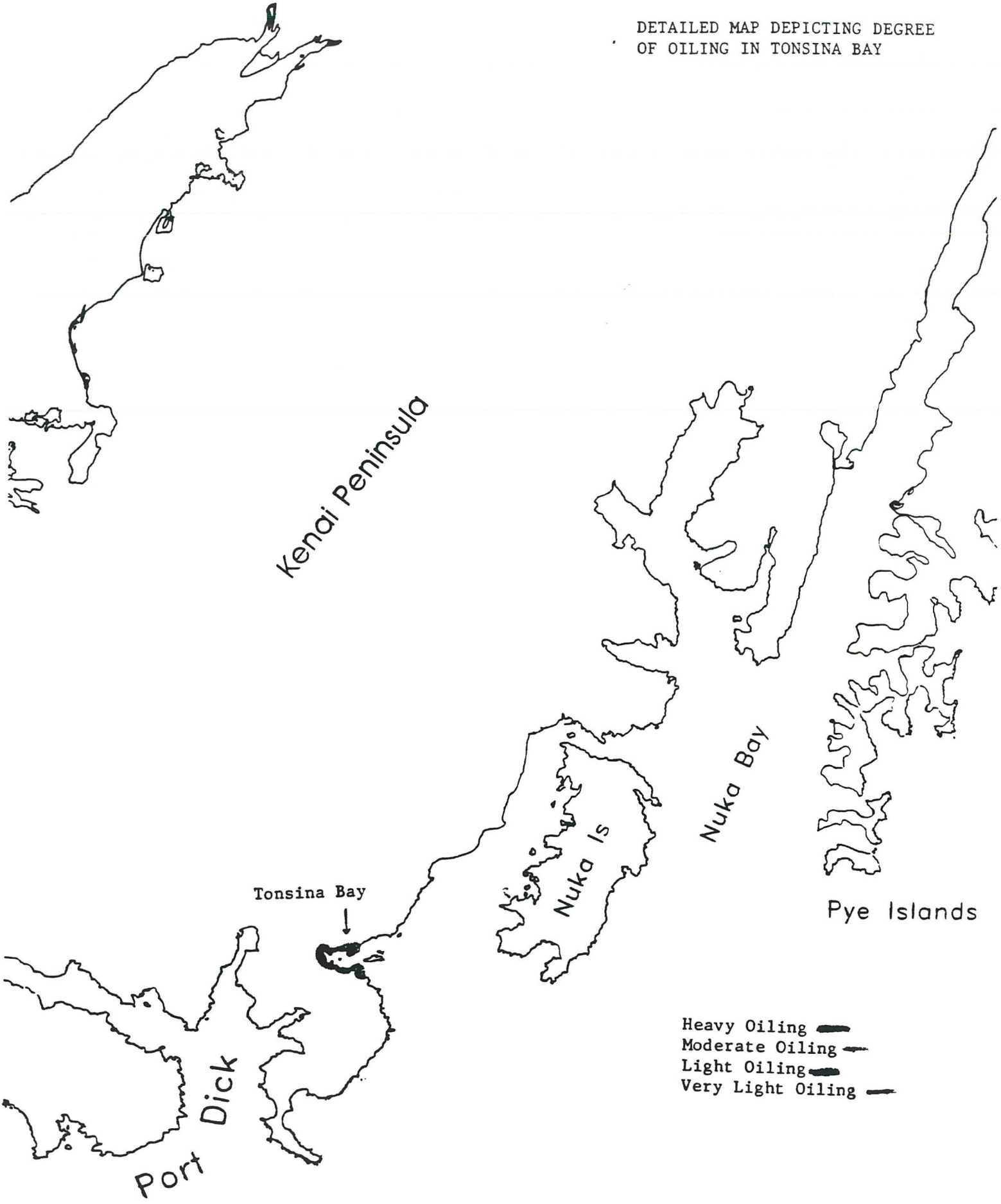
DETAILED MAP DEPICTING DEGREE
OF OILING IN BAY OF ISLES



SOURCE: Alaska DEC oil spill maps


162°
156°
Bethel
Anchorage
Kenai
Dillingham
ALASKA
Kodiak
58°
56°
Cold Bay
100
200
1000
2000
KENAI PENINSULA -- FOR DETAIL OF INDICATED AREA SEE DETAIL MAP OF TONSINA BAY
50°
0
100
200
300
400
Km
Mi
0
100
200
300
400

DETAILED MAP DEPICTING DEGREE OF OILING IN TONSINA BAY



SOURCE: Alaska DEC oil spill maps

ATTACHMENT A

RESTORTION DATA FORM

ATTACHMENT B

SITE MONITORING FORM

EXAMPLE TIDAL MARSH RESTORATION DATA FORM

Restoration Assessment

Investigators Name _____
Date _____
Time _____
Location _____
Segment Number/ID _____

1 Site Description:

2 Extent of original oiling (Based on oil map)

_____ moderate Areal percent _____

_____ heavy

3 Extent of living/dead vegetation:
i) Apparent Cover: _____%

ii) _____% aboveground biomass

iii) Belowground biomass present beyond limit of aboveground biomass?
_____ Y _____ N

if yes, extent of total marsh is _____%;
location _____

4 i) Location/tidal zone of each treatment area
Permanent reference point location (landmark) _____

Distance from reference point (yards)		Elevation	
#1T	_____	#1T	_____
#2T	_____	#2T	_____
#3T	_____	#3T	_____
#4T	_____	#4T	_____
#5T	_____	#5T	_____
#6T	_____	#6T	_____

ii) substrate type (S=Sand, SH=Shale, R=Rock/Cobble, M=Mud)

#1T	_____	#4T	_____
#2T	_____	#5T	_____
#3T	_____	#6T	_____

iii) Location/tidal zone of each control area

Distance from reference point (yards)		Elevation	
#1C	_____	#1C	_____
#2C	_____	#2C	_____
#3C	_____	#3C	_____

iv) Salinity _____‰

5 Approximate area (m^2) to be restored per species:

List: species area

_____

_____

_____

6 Number of transplants needed:
9 holes/m^2 @ 3 plants/hole

	species	number
i)	_____	
ii)	_____	
iii)	_____	
iv)	TOTAL	_____

7 Amount of fertilizer needed: _____ lbs.

# of plants (6 iv) x .066 lbs. = lbs. of fertilizer needed

8 Vegetative Donor Site:
i) Proximity to restoration site (approximate miles)_____

ii) Size (m^2) _____

iii) Donor site identification number _____

9 Comments:

10 Soil Analyses (record shipping information on reverse side):
Soil sample taken? _____ Y _____ N

i) If yes, number of samples (including duplicates) _____

ii) I.D. numbers _____

iii) Method of storage _____

11 Oil characteristics at the site:

i) surface _____

ii) subsurface _____

iii) asphalt _____

iv) sheen _____

EXAMPLE TIDAL MARSH SITE MONITORING DATA FORM

Investigators Name _____
Date _____
Time _____
Location _____
Segment Number/ID _____

1 Restoration method used:
i) _____ fertilization

ii) _____ transplant/fertilize

iii) Date treated _____

2 Species used for each treatment plot:

List:

#1T _____ #4T _____
#2T _____ #5T _____
#3T _____ #6T _____

3 Living/dead vegetation cover per treated and control areas:

#1T _____ %
#2T _____ %
#3T _____ %
#4T _____ %
#5T _____ %
#6T _____ %

#1C _____ %
#2C _____ %
#3C _____ %

4 Substrate samples collected for oil/nutrient analysis (Y or N)

	Oil	Nutrient
#1T		
#2T		
#3T		
#4T		
#5T		
#6T		

5 Apparent vigor

	1T	2T	3T	4T	5T	6T	1C	2C	3C
i) Vigorous[1] (%)									
ii) Healthy[2] (%)									
iii) Low[3] (%)									
iv) Poor[4] (%)									
v) Dying[5] (%)									

6 Comments:

Key: [1] (healthy color, >80% cover)

[2] (healthy color, 20-80% cover)

[3] (healthy color, <20% cover)

[4] (unhealthy color)

[5] (unhealthy color - brown stems; sparse cover)

Resumes of PWS/GOA Restoration Team Members

Clarence Arthur Callahan

24143 Henderson Road
Corvallis, Oregon 97333

BIRTH DATE: September 29, 1943 PHONE: Home: 503/929/5955
Office: 503/757-5764
Message: 503/757-4600

PHYSICAL CHARACTERISTICS: 5'9", 195 lbs.
HEALTH: Excellent

EDUCATION:

B.S. 1966 University of Southwestern Louisiana
Zoology major, Chemistry minor
Lafayette, Louisiana

M.S. 1968 Auburn University
Entomology major, Zoology minor
Auburn, Alabama

TITLE OF THESIS: The Effects of Photoperiod and Light Intensity on Oviposition of the Southwestern Cornborer, Diatraea grandiosella Dyar.

Ph.D. 1976 Purdue University
Entomology major, Invertebrate Ecology minor
West Lafayette, Indiana

TITLE OF DISSERTATION: An Evaluation of Nematode Community Structure as a Method for Quantifying and Interpreting Ecological Changes in Water Resource Environments.

EXPERIENCE:

a. I am presently the acting Team Leader for the Ecological Site Assessment Team and the Work Assignment Manager for the Site Assessment Work (Superfund support) being completed by the on site contractor, METI. This involves the management of the work assignment and the work plan for as many as 11 personnel, some of whom have split duties, but are essentially working on some aspect of the Superfund support group.

b. I am presently presently Project Leader for two projects; 1) Superfund Site Assessment/Technical Assistence activities; and 2) Ecological Site Restoration Research.

c. I have served as project officer for cooperative agreements and contract work concerning research at Superfund Sites.

d. I have served as a member of the Organizing Committee for an International Workshop for Earthworm Ecotoxicology that was held in Sheffield, England, April, 1991.

e. I am serving as a member of the Organizing Committee for the 1991 Society of Toxicology Annual Meeting in Seattle, WA.

f. The EPA's research effort at Oregon State University's Oak Creek Laboratory of Biology was coordinated by me. This research effort involved studying the impact of sedimentation on fish production. The research resulted in a journal article and a colleague completing the requirements for a Master's Degree in Fisheries Biology from Oregon State University.

g. A Hazardous Materials Assessment Team (HMAT) effort to assess the impact of hazardous materials on soil organisms was conceived and carried out under my direction. This task resulted in a revision of the standard method of the Organization of the European Economic Community protocol for the Laboratory Earthworm Soil Test. The new method was refined in an intensive extramural and in-house research effort involving testing pure chemicals as well as soil samples from Superfund Sites. As a result of this work, the laboratory method is now used routinely for laboratory assessments and further work resulted in the development and testing of a field protocol.

PROFESSIONAL EXPERIENCE:

a. Presently, Research Biologist GS-401-13, Ecotoxicology Branch, Ecological Site Assessment Team, USEPA Corvallis Environmental Research Laboratory.

b. Research Biologist GS-401-12, 1983-1991, Hazardous Materials Assessment Team, USEPA Corvallis Environmnetal Research Laboratory.

c. 1986, Fall Quarter, Guest Lecturer, Entomology Department. Guest Lecturer - A graduate level seminar was developed and taught by me in the Department of Entomology at Oregon State University on the subject of the Impact of Pesticides on Non-target Species. Class presentations were made on the biology, ecology, and chemical impact to the organism, population, and the community in the context of pesticide applications for control of pest organisms.

d. 1979-1981, Aquatic Biologist GS-401-11, Freshwater Division, Lake Restoration Team, USEPA Corvallis Environmental Research Laboratory.

Collected data for evaluation as part of the Lake Restoration Studies in order to determine the effectiveness of restoration methods.

e. 1979, Part-time Instructor of Biology, Chemeketa Community College, Salem, Oregon.

The lecture portion of general biology was taught to students at the Men's and Women's Oregon Correctional Facility, Salem, Oregon. This introductory biology course was part of the core curriculum for nurses training in the State Certification Program.

f. 1976-1979, Aquatic Biologist GS-401-12, Freshwater Division, Ecology Branch, USEPA Corvallis Environmental Research Laboratory.

Worked as stream biologist and fisheries biologist in studies dealing with sedimentation impact on fisheries production utilizing both laboratory and field studies.

g. 1973-1976, Aquatic Biologist GS-401-12, Freshwater Division, Lake Survey Branch, USEPA Pacific Northwest Water Laboratory, Corvallis.

As one of three principal authors, I helped to write 600+ water quality reports for the lakes that were surveyed by the National Eutrophication Survey Team. Part of my responsibility included the supervision of two programmers who provided computer support for data storage, retrieval, and manipulations of all data sets.

h. 1972-1973, Limnologist GS-401-11, National Lake Survey Team, USEPA, Las Vegas, NV.

Two helicopters were used by a field sampling team supporting the National Eutrophication Survey Program. I directed daily field sampling operations for one of the helicopters, determining the number, location, and depth of samples taken in all lakes surveyed. A sampling technician and two chemical technicians were under my supervision.

PROFESSIONAL ACTIVITIES AND RECOGNITION:

a. Honors and Awards:

1. Gold Medal Award, U.S. Environmental Protection Agency
2. Elected to Sigma Xi, Oregon State University Chapter
3. Certificate of Achievement, U.S. Environmental Protection Agency, 1977 and 1980.
4. Certificate of Appreciation for Extraordinary Volunteer Service as an Advanced Placement Biology Mentor for Corvallis High School Students, 1988-1989 School Year.

b. Society Membership:

Sigma Xi Society, Oregon State University Chapter
American Fisheries Society
Ecological Society of America
Society of Environmental Toxicology and Chemistry
Northwest Scientific Association

c. Professional Registration:

1. Certified Fisheries Scientist, No. 1319, 1980
2. Certified Open Water SCUBA, 1983

CIVIC AND VOLUNTEER ACTIVITIES:

a. Cub Scouts. I served as Cub Master and Den Leader for three years that my son participated in the Scouts.

b. School District 509J. I served on the Science Textbook and Curriculum Committee, during which time I was the leader in organizing a local Science Fair.

c. American Youth Soccer Organization. I volunteered as a coach

for three years for 11, 12, and 13 year-old participants. I now serve as a Board Member in the capacity of Fields Coordinator. We administer a program for about 1500 youths during the year.

I organized and directed an Indoor Soccer Program for high school players during 1986 and 1987.

d. I have served as an Equal Employment Opportunity Counselor for the period 1980-1988.

PUBLICATIONS LIST

Clarence A. Callahan

Callahan, C.A. 1988. Earthworms as Ecotoxicological assessment tools. In: C.A. Edwards and E.F. Neuhauser (Eds) Proceedings of the International Conference on Earthworms in Waste and Environmental Management, July, 1984. Cambridge, England, pp295-301, SPB Academic Pub., The Hague, The Netherlands.

Callahan, C.A., L.K., Russell, and S.A. Peterson. 1985. A comparison of three earthworm bioassay procedures for the assessment of environmental samples containing hazardous wastes. Biol. Fert. Soils Vol. 1 pp 195-200.

Callahan, C.A., C.A. Menzie, D.E. Burmaster, D.C. Wilborn, and T.Ernst. 1991. On site methods for assessing chemical impact on the soil environment using earthworms: A case study at the Baird & McGuire Superfund Site, Holbrook, MA. Vol 10(6) May, 1991.

Bromenshenk, J.J. and C.A. Callahan (MS) Protocols for Exposure and Toxicity Screening of Hazardous Waste Sites with Honey Bees (Apis mellifera L.). In preparation for publication.

Bromenshenk, J.J. and C.A. Callahan. 1990. Site Specific and Regional Monitoring with Honey Bees: A Case Study Comparison. Presented at Ecological Indicators, Fort Lauderdale, FL, October 15, 1990.

Drewes, C.D., C.A. Callahan and W.M. Fender. 1983. Species Specificity of Giant Nerve Fiber Conductivity Velocity in Oligochaetes. Can. Jour. Zool. 61:2628-2694.

Drewes, C.D., E.P.Vining, and C.A. Callahan. 1984. Non-invasive electrophysiological monitoring:A sensitive method for detecting sublethal neurotoxicity in earthworms. Env. Tox. and Chem. Vol 3.pp 599-607.

Drewes, C.D., M.J. Zoran, and C.A. Callahan. 1987. Sublethal neurotoxic effects of the fungicide benomyl on earthworms (Eisenia foetida). Pest. Sci. Vol. 19 pp 197-208.

Drewes, C.D., E.P. Vining, and C.A. Callahan. 1988. Electrophysiological detection of sublethal neurotoxic

effects in intact earthworms. In: C.A. Edwards and E.F. Neuhauser (Eds) Proceedings of the International Conference on Earthworms in Waste and Environmental Management, July, 1984. Cambridge, England, pp356-366, SPB Academic Pub., The Hague, The Netherlands.

E.F. Neuhauser and C.A. Callahan. 1990. Growth and reproduction of the earthworm, Eisenia fetida exposed to sublethal concentrations of organic chemicals. Soil. Biol. Biochem. Vol. 22(2)pp175-179.

Callahan, C.A., M.A. Shirazi and E.F. Neuhauser. (in review). An evaluation of Relative Toxicity to Earthworms. planned for submission to SETAC by March, 1991.

Callahan, C.A. (on-going research) The performance of the 28 Day Earthworm Laboratory Test for two Species, Eisenia fetida and Lumbricus terrestris. (Scheduled for completion 10/1/91).

Menzie, C.A., D.E. Burmaster, and J.S. Freshman, and C.A. Callahan. 1991. Assessment of methods for estimating ecological risk in the terrestrial component: A case study at the Baird & McGuire Superfund Suite, Holbrook, MA. Soc Env Tox and Chem, Vol 6(10), May.

Publications Resulting from
Cooperative Agreements

Clarence A. Callahan, Project Officer

Bromenshenk, J.J. and N. Lockwood-Ogan. 1990. Sonic Digitizer as an Alternate Method to Asses Honey Bee (Hymenoptera:Apidae) Colony Dynamics. J. Econ. Entomol. 83(5):1791-1794.

Bromenshenk, J.J., J. Doskocil, G.J. Olbu, G. Degrandi-Hoffman, and S.A. Roth. 1991. PC BEEPOP, An Ecotoxicological Simulation Model for Honeybee Populations. Enviro. Toxicol. Chem. Vol.10, pp547-558.

Bromenshenk, J.J. 1990. PC BEEPOP (Personal Computer Honey Bee Population Dynamics Model) for Ecological Assessments User's Guide. EPA Cooperative Agreement CR-814456. USEPA, ERL-Corvallis, 63pp and three accompanying 5 1/4" diskettes.

Bromenshenk, J.J. and C.A. Callahan (MS) Protocols for Exposure and Toxicity Screening of Hazardous Waste Sites with Honey Bees (Apis mellifera L.). In preparation for publication.

Bromenshenk, J.J. and C.A. Callahan. 1990. Site Specific and Regional Monitoring with Honey Bees: A Case Study Comparison. Presented at Ecological Indicators, Fort Lauderdale, FL, October 15, 1990.

Burmaster, D.E., C.A. Menzie, J.S. Freshman, J.A. Burris and S.R. Drew. 1991. Assessment of methods for estimating ecological risk in the aquatic component: A case study at the Baird & McGuire Superfund Site, Holbrook, MA. Vol 10(6) May, 1991.

Drewes, C.D., 1984. Escape reflexes in earthworms and other annelids. In: Neural Mechanisms in Startle Behavior. R.C. Eaton, Ed. Plenum, NY. pp43-91.

Drewes, C.D., C.A. Callahan, and W.M. Fender. 1983. Species specificity of giant nerve fiber conductivity velocity in oligochaetes., Can. J. Zool. 61:2628-2694.

Drewes, C.D., and E.P. Vining. 1984. In vivo neurotoxic effects of dieldrin on giant nerve fibers and escape reflex function in

the earthworm, Eisenia foetida. Pesticide Biochem. Physiol. 22:93-104.

Drewes, C.D., E.P. Vining and C.A. Callahan. 1984. Non-invasive electrophysiological monitoring: A sensitive method for detecting sublethal neurotoxicity in earthworms. Environ. Toxicol. and Chemistry 3:325-334.

Drewes, C.D., E.P. Vining and C.A. Callahan. 1988. Electrophysiological detection of sublethal neurotoxic effects in intact earthworms. In: Proceedings of International Conference on Earthworms in Waste and Environmental Management, July 1984, Cambridge, England.

Drewes,C.D., M.J. Zoran and C.A. Callahan. 1987. Sublethal neurotoxic effects of the fungicide benomyl on earthworms (Eisenia fetida). Pest. Sci.197-208.

Fender, W.M. 1985. Earthworms of the western United States.. Part 1. Lumbricidae. Megadrilogica Vol.4(5) pp93-129.

Fender, W.M. and D.McKey-Fender. (MS). Earthworms of the Western United States, Part II. Native species and miscellaneous exotics.

Menzie, C.A., D.E. Burmaster, and J.S. Freshman, and C.A. Callahan. (in review) Assessment of methods for estimating ecological risk in the terrestrial component: A case study at the Baird & McGuire Superfund Suite, Holbrook, MA. Submitted to Society of Toxicology and Chemistry.

Neuhauser, E.F., M.R. Malecki, and R.C. Loehr. 1984. Growth and reproduction of the earthworm, Eisenia foetida after exposure to sublethal concentrations of metals. Pediobiologia 27,89-97.

Neuhauser, E.F., P.R. Durkin, D.L. Milligan, and M. Anatra. 1986. Comparative toxicity of ten organic chemicals to four earthworm species. Comp. Biochem. Physiol. Vol. 83C. No.1, pp 197-200.

Neuhauser, E.F., R.C. Loehr and M.R. Malecki. 1986. Contact and artificial soil tests using earthworms to evaluate the impact of wastes in soil. In: Hazardous and Industrial Solid

Waste Testing: Fourth Symposium, ASTM STP886. J.K. Petros, Jr. and R.A. Conway, (Eds). American Society for Testing and Materials. Philadelphia, 1986, pp192-203.

Neuhauser, E.F., R.C. Loehr, D.L. Milligan, and M.R. Malecki. 1985. Toxicity of metals to the earthworm, Eisenia foetida. Biol. Fert. Soils. 1:149-152.

Neuhauser, E.F., M.R. Malecki, and R.C. Loehr. 1983. Methods using earthworms for the evaluation of potentially toxic materials in soils. In: Second Annual ASTM Symposium on Testing of Hazardous and Industrial Solid Waste. R.A. Conway and W.P. Gulledge (Eds.). Hazardous and Industrial Solid Waste Testing. ASTM STP805. ASTM, Philadelphia, PA, pp313-320.

Neuhauser, E.F., R.C. Loehr, D.L. Milligan, and M.R. Malecki. 1985. The toxicity of selected organic chemicals to the earthworm, Eisenia foetida. J. of Environ. Qual. 14:383-388.

Neuhauser, E.F., M. Malecki, and Z.V. Cukic. 1985. Metal content of earthworms in sludge amended soils: Uptake and loss. In: International Conference on Heavy Metals in the Environment, Ed: T.D. Lekkas, Athens, Greece. 9/85.

Vining, E.P. and C.D. Drewes. 1985. Functional connections are established between giant nerve fibers in grafted earthworms. J. Exp. Zool. 233:121-125.

Zoran, M.J.,T.J. Heppner and C.D. Drewes. 1986. Teratogenic effects of the fungicide, benomyl on posterior segmental regeneration in the earthworm, Eisenia fetida. Pestic. Sci. 17, 641-652.

Mostafa A. Shirazi

Education:

Ph.D. Mech. Enginr. University of Illinois, 1966.
M.S. Mech. Enginr. University of Washington, 1960.
B.S. Mech. Enginr. Clif State University, 1959.

Professional Interests:

Fluid dynamic, Heat transfer, Particle motion, Turbulent flow.

Thermal pollution, Modeling heated water discharge in rivers, lakes and the ocean, Physical modeling and prediction of mixing zone for ambient discharge.

Non-point source pollution, Watershed classification and regionalization, Characterization of spawning substrate for salmonids and assessment of biological impact of nonpoint source sedimentation.

Hazardous waste characterization regionalization and GIS mapping of impacted resources.

Soil texture development, soil microcosm analysis, functional capacity of microbial respiration.

Toxicity of single and mixtures of chemicals to laboratory organisms, dose-time-response analysis, structure activity analysis.

Professional Experience:

EPA in Corvallis since 1969 (GS-13,14,15):
Participated in diverse environmental programs with emphasis upon modeling, interpretation, integration and utilization of biological observations to environmental management.

Hercules Inc. 1966-1969
Gasdynamic of rocket propulsion, two-phase flow and nozzle design.

Boeing Co. 1960-1961
Computation and analysis, heat transfer and gas dynamics.

Safa Shirazi
Jan 1989

Update: Winter 1984 - Winter 1989

Period (approximate)	Supervisor
winter 1984 - fall 1985	Spence Peterson
fall 1985 - fall 1986	Phil Larson/Peterson
fall 1986 - fall 1987	John Emlen/Kibby
fall 1987 - winter 1987	Hal Kibby
winter 1988 - present	Larry Kapustka/Kibby

ECOLOGICAL SITE ASSESSMENT TEAM

Overview

During the early part of this period (see also 1981-84 update) I was a member of the hazardous waste team dealing with "Superfund" program. The support for Superfund had taken a sharp turn downward, leading to reduced funds and a change in plans. The broad regional approach initially envisioned by the team was now de-emphasized in favor of a more expedient approach using laboratory bioassay tests with contaminated samples of soils from actual hazardous waste sites. The initial aim of the regional approach was to classify attenuating capacity of soil environments in different biogeoclimatic zones of the US with the help of laboratory bioassays and biological tests in the field. The scale-down to just soils and bioassays with laboratory organisms decoupled the problem for a while. Some results of fundamental work on soils was published (see Soil Texture below) and results of several program elements leading to an integrated regional approach were also published to facilitate returning back to the problem in the future (see Land Classification below).

Because of the strong program interest on laboratory tests, my efforts were directed to development of fundamental work in integration, interpretation and use of laboratory toxicity data. This work dovetailed neatly into a newly developed risk assessment program in Ecotoxicity Branch and later, the Plant Team, to which I now belong. The Plant Team is assigned a broad range of environmental risk assessment problems of exposure to hazardous waste and toxic chemicals in response to EPA programs in Superfund, RCRA, and Toxic and Pesticide Control Acts. They provide the opportunity anew to reconsider the work on regional approach in environmental management.

The gap between laboratory toxicity tests and environmental risk of exposure to a mixture of chemicals remains wide. The gap will not shrink to zero overnight and the answer does not lie in laboratory tests alone, in field tests alone, in regional analysis alone, etc., etc. A concerted effort in all areas must be made, taking one step at a time. The problems have much longer memory of persistence than the patience of one or two persons, but they will give way to resolved and dedicated people.

Soil Texture

The following is a quotation from a textbook on soil physics by Gaylon S. Campbell (p. 9, Elsevier, New York 1985): "Soil scientists use textural information to make qualitative judgement about a number of other physical properties, but until recently, quantitative use of textural data has been difficult....Shirazi and Boersma (1984) recently produced a new texture diagram that is much more useful for obtaining physical data from soil texture...." This is a reference to publication no. 49 which was developed as a part of a program for mapping the vulnerabilities of soils to the effects of hazardous waste contamination and for classifying soil types when used in laboratory bioassay tests. This work was expanded further (publication nos. 58, 63) and the work attracted intense international commentary (publication no. 65). The attachments a and b are examples of reactions to the new soil texture, one in education and a second in environmental management. See also soil-microbe related work(publication nos. 50,54).

Land Classification

Hazardous waste sites often release toxic chemicals into the environment. Natural environments have differing potentials for attenuation of harmful effects of toxic waste. A realistic management approach works within the limits of these regional potentials. The climate, geology, and biota together determine the attenuating potential of the environment and when mapped, they delineate boundaries of ecologically uniform and distinct regions. The feasibility for application of uniform management approaches relative to control of hazardous waste and toxic chemicals can be tested within mapped homogeneous regions.

Land classification of Southeastern United States was used in one application (publication no. 48) to select hazardous waste sites for indepth ecological studies in a cooperative agreement with Florida State University (publication no. 56). In a separate study (publication no. 59) Land Classification of the Conterminous United States was mapped for classifying results of bioassay tests. As an example, parathion was shown to have different toxicity on field crickets. The toxicity correlated with the attenuating capacity of soil environments.

These studies in land classification were computerized and were fully quantitative. They were undertaken with limited resources and long before the laboratory aquired a geographic information processing system. The home-made programs were implemented on a PDP-11 general purpose computer and the maps were plotted via an aging Calcomp plotter on that system. Several classes of thematic mapped information were overlayed using a computer to define land classes. A unique feature of the classification scheme was to regroup existing mapped data at the

outset before overlay. The regrouping of the original maps into fewer, ecologically-related map features clearly identified the assumptions used and enabled a quantitative analysis and interpretation of land classes in a composited map (see attachment c).

Biological mode of response in risk analysis

Toxicity tests are conducted for determining biological effects of pesticides, toxic substances and hazardous waste in response to related legislation for the environmental protection and the protection of human health. The majority of tests are conducted with laboratory animals and plants using single chemicals and single species and acute dose levels. In a real environment we often find mixtures of chemicals at low levels impacting a community of organisms. Therefore, the problem begins at a conceptually difficult starting point.

If somehow we double the number of laboratory tests and conduct many more field tests with all kinds of chemicals, we will still have problems making sense out of the results and would have to rush to new ~~data sets~~ tests to answer apparently a new problem. I feel that this is what has actually been happening.

There are more data collected already than a whole institute can produce in several lifetimes. On the other hand, if we were to devote equal time to testing as to interpreting or enough time to interpreting the tests we just finished before we think of a new one, the cycle would slow down a bit and we would live to deserve the title of (conservative) environmentalists!

Where the passion of other scientists has been in learning by testing, my passion has been the learning from what others have tested. I have always loved this symbiotic existence in environmental science and I find the field of toxicology to be a good place to bring new interpretations to abandoned or half-used data. This can be done while fully remaining accountable to program needs and producing innovative procedures for interpretation of results, design of new experiments and facilitating environmental risk assessment. I will outline analyses of several problems, all intended to extract explanation while integrating data for environmental risk assessment.

Plant bioassay: The first example is the analysis of interlaboratory crop root data consisting of 560 dose-response tests from 7 different laboratories, 5 species and 8 chemicals.

The interpretation of crop root results are made difficult simultaneously by 1) experimental variability at low dose; 2) the possibility of stimulatory response at a low dose; and 3) the desire to obtain a clear picture of the dose-response relationship at all dose levels, including the commonly used dose at 50% relative response level (R=0.5). The relative response at zero dose is by assumption equal to unity (R=1.0 at D=0). At a

low dose level the response may exceed unity, and then continue to drop at higher dose levels thereafter. This stimulatory response may be due to experimental error or due to some biological reasons. Experimental errors are present at all dose levels and the response may exceed unity at any dose when the noise-to-response ratio is large. Typically, the response is very weak at a low dose and the noise becomes more of a problem at that level. There would be no problem if we ignored the possibility of a stimulatory response. Since many probit analyses cannot handle a relative response that is greater than unity, the data points are simply ignored. Also, since many end points are based on 50% relative response, only the central portion of the data are used, leaving the sleeping dogs lie.

I have examined this problem in several ways. Publication no. 53 is an example of one approach. Although not a full solution, this is an example of an exploratory approach attempting to raise the problem of analyzing single tests to a higher level and attack it there first. It shows that even if we anticipate biologically meaningful stimulatory response at a low dose, the stimulatory portion is hidden in the noise and cannot be pulled out without additional analysis, redesign of test procedure, or both.

Sublethal effects: The structure of the dose-response curve at a low dose level is particularly important when dealing with interpretation of sublethal effects. One example is the response of the immune system to extremely low levels of pesticides. I was exposed to this problem as a guest scientist at the University of Wisconsin (see attachment d). Prof. Hinsdill was finishing a series of tests of low levels of Aldicarb in the drinking water of mice. In one series of tests he could see a clear presence of immune suppresion but not in another similar series of tests. I have examined several sets of data and linked the noise to the variability of the animal population used in these tests.

The conventional approach smoothes over the variability by averaging the response for the animals in the treatment and compares it with the average response of animals in the control. In a new approach I went after the variability as a part of the problem to be solved. I compared the relative response of every individual animal in a treatment with all animals in a control. This produced a distribution whose mode was taken to be the most representative response for a treatment. This approach recovers the immune suppression in the series of tests with noise (publication no. 66).

Why only LC50?: The slope of the response curve varies continuously along the dose axis. It varies differently for different chemicals and organisms. The structure of a full dose-response curve determines the mode of biological response. Two different chemicals may have, for example, identical LC50 numbers but different slopes at LC50. As a result, the response to incremental change at LC50 may be substantially less severe

for one, imposing much smaller environmental risk than the other. The full structure of the dose-response curve can be summarized via appropriate scalers using all data points in a test. The practice of using a single LC50 from all data points in a test ignores the utility of other information for environmental risk assessment and management. Publication nos. 60,61 and 64 introduce the use of Weibull function to dose-response data, thereby condensing an entire form and scale of a dose-response curve into two numbers calculated directly from data. The approach is applied to a diverse group of data showing utility of the approach in interpretation of results and comparison of relative toxicities.

The time-response information is often an integral part of any toxicity test, but seldom examined in depth beyond acute, chronic, 24 hr, 96 hr, etc. The duration of exposure is of equal importance to the level of the dose. The environmental risk of exposure cannot be determined without both information, but the analysis for evaluating ~~from~~ the data is wanting. The structure of a dose-response curve and time-response curve together determine the mode of biological response of an organism to a chemical. Publication no 62. extends the Weibull function to full consideration of the time component of toxicity. I have responded to more than 150 requests worldwide for these papers in just one year.

QSAR and biological response: The above series of papers are interrelated. They address simultaneously the problem of experimental variability and the biological mode of response. The classification of biological mode of response based on actual experimental data, testing whole organisms is a useful complement of the classification of toxicity of chemicals based on the structure of a chemical. Publication no. 67 develops a comprehensive model of classification of mode of biological response for 470 different chemicals using full dose-time-response tests with fathead minnows. The next research phase will be devoted to the analysis of mixtures using the model.

Other works

1) During this period I refereed numerous manuscripts for publication in scientific journals, including ASTM, Soil Science, Soil Science Society of America, Envirionmental Management, Archive of Environmental Toxicity and Contanimation (attachments e,f,g)

2) I continue to provide expert assistance to EPA on my past research works, for example, in sedimentation (attachment h) and thermal plumes (attachment i) and I have responded to several requests in the past to permit the use of plume work in textbooks. Attachment j is an example of a recent request for this 15 years old work.

3) I have been re-evaluated by the Graduate School of the Oregon State University for my scientific work during the past 5 years and was approved for retaining my courtesy position (attachment k).

4) I contribute to and learn from the participation in the advisory commission of the City of Corvallis Watershed. I learn about the problems of water supply, timber revenue, habitat for research and for the spotted owl (attachment l)!

5) I owe a debt of gratitude to my major professor, a mechanical engineer at the University of Illinois. I visited him after 20 years at his retirement. He has left his mark in my works outside his own field (attachment m).

6) I have received a 20-year pin for a continuous government service at EPA. I am, indeed, indebted now to many more who helped me to be the spokesman for the above works.

SF 171

Item 22 A:

SUMMARY OF PUBLICATIONS AND PRESENTATIONS

PeerReview Journals:

Item 10,18,26,29,32,40,41,43,44,47,
48,49,50,52,53,54,59,60,61,62,63,64,65
66,67,74,75,76

Workshop and Symposium Presentations:

Items: 2,3,5,8,16,17,21*,23*,24,25**,27*,
31*,33,35*,39,42,45,46,51,52,56,57,58,68,69,70,71
72,73,73a,77,78

Others, Government and Private:

Items: 1,4,6,7,8,9,11,12,13,14,15,19,20,22
28,30,34,36,37,38,55

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* Invited
** Session Chairperson

PUBLICATIONS OF

MOSTAFA A. SHIRAZI

1. Shirazi, M. A. 1967. On the Motion of Small Particles in a Turbulent Fluid Field, Ph.D. Thesis, University of Illinois, Urbana, Illinois. 131 pp.

2. Shirazi, M. A., B. T. Chao, and B. G. Jones. 1967. On the Motion of Small Particles in a Turbulent Fluid Field. Developments in Mechanics, Proceedings of the Tenth Midwestern Mechanics Conference. p. 1179, Vol. 4.

3. Jones, B. G., B. T. Chao, and M. A. Shirazi. 1967. An Experimental Study on the Motion of Small Particles in a Turbulent Fluid Field Using Digital Techniques for Statistical Data Processing. Developments in Mechanics, Proceedings of the Tenth Midwestern Mechanics Conference. p. 1249, Vol. 4.

4. Shirazi, M. A. Hydrodynamics of Sewage Filtration. Final Report by Hercules Incorporated for Federal Water Pollution Control Administration, Contract No. 14-12-39 (Staff Report).

5. Shirazi, M. A. 1968. The Effects of Closure Ejection of Sprint Nozzle Structural Integrity, Third International Congress for Rocket Propulsion and Guidance, American Institute of Aeronautics and Astronautics, Vol. 1, p. 31. (Confidential).

6. Shirazi, M. A. 1968. Performance of Oval and Pinched Nozzles, Final Report prepared for Applied Physics Laboratory, John Hopkins University, Contract 271803, Feb. 1968. (Confidential).

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RPWG
(A)

QUALITY ASSURANCE PROJECT PLAN
for
Feasibility of Restoring the
Bay of Isles and Tonsina Bay in
Prince William Sound and the Gulf of Alaska

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for
Feasibility of Restoring the
Bay of Isles and Tonsina Bay in
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QUALITY ASSURANCE PROJECT PLAN
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4.0 INTRODUCTION

A quality assurance project plan (QAPP) is designed to ensure that all environmentally-related data collected will meet project data quality objectives (DQOs), and be scientifically sound, legally defensible, and of known and documented quality. This plan follows the guidance for preparing QAPPs provided by the Environmental Protection Agency's (EPA's) Quality Assurance Management Staff in the document "Interim Guidelines and Specifications for Preparing Quality Assurance Project Plans (US EPA, 1980). QAPPs are considered to be stand-alone documents that fully explain the methods and activities to be implemented for data collection. Analytical methods and standard operating procedures (SOPs) are included as appendices to the QAPP.

In March 1989 the *Exxon Valdez* ran aground on Bligh Reef in Prince William Sound, spilling approximately 11 million gallons of Prudhoe Bay Crude Oil into the water and impacting over one thousand miles of coastal resources in the Prince William Sound (PWS) and the Gulf of Alaska (GOA). The *Exxon Valdez* oil spill (EVOS) affected the region's environmental habitat, including both floral and faunal populations, as well as recreational, educational, and aesthetic attributes.

According to Gundlach and Hayes (in Ganning et al., 1984), tidal marshes have been classified as the most sensitive shore-type to oil pollution. It has been estimated that 2-20 years are required for tidal marshes to recover naturally (Cairns and Buikema, 1984; RPWG, 1990a; 1990b). Oil is rapidly buried in marshes because they are low energy systems, and degradation is limited under the anaerobic conditions found in these environments (Cairns and Buikema, 1984).

Natural marsh recovery begins when oil toxicity is reduced to a point that can be tolerated by recolonizers (Baker et al., 1990a; 1990b). Full tidal marsh recovery hinges on reduction in oil toxicity; availability of propagules; stability of sediments; and biotic interactions (Getter et al., 1984). Restoration activities in heavily oiled marshes may be expected to require both substantial effort and extended time periods. The presence of oil in high concentrations at a site may complicate restoration efforts, and regrowth in these areas may occur slowly, if at all.

There are two tidal marshes, the Bay of Isles and Tonsina Bay, that are still heavily oiled and lacking in vegetation. A qualitative survey conducted over 4 days in August of 1990 noted (1) heavy effects from residual oil (approximately one acre of mixed Carex and Triglochin) and suspected effects to 1/4 to 1/2 acre of Zostera at the Bay of Isles, and (2) extensive effects to Puccinellia from residual oil at Tonsina Bay; Glaux at higher elevations in this marsh was not affected. Due to these effects on the marshes from the oil, the Bay of Isles and Tonsina Bay require restoration. See Attachment A of the project proposal for maps of Prince William Sound and the Gulf of Alaska, as well as detailed maps of the Bay of Isles and Tonsina Bay showing oiled areas as based on the oil spill maps.

The goal of this feasibility study is to determine whether or not vegetation can be enhanced and/or re-established at the Bay of Isles and Tonsina Bay restoration sites, two tidal marshes known to be heavily impacted by oil.

5.0 PROJECT DESCRIPTION

The project has the following objectives:

- to quantitatively determine the degree of revegetation success (proportional survival/plot) and relate to crude oil degradation patterns using spatial analysis techniques,
- at the Bay of Isles test site, increase the rate of ground water discharge to a small section of the wetland in order to determine if sediment characteristics improve more rapidly where the rate of ground water discharge has been increased than in areas where the rate of ground water discharge is normal,
- to demonstrate whether revegetation success rates improve in areas where the rate of ground water discharge has been increased than in areas where the rate of ground water discharge remains normal.

- to monitor overall site revegetation success on an annual basis. On plots where there is no revegetation success, replant at similar densities in the succeeding year(s).

Site Restoration

Site restoration will consist of identifying donor sites, conducting restoration trials, and conducting site monitoring. The first annual planting will occur in the spring of 1991. Stands will be established using species native to Prince William Sound and the Gulf of Alaska. At each site, twenty-four 10 m^2 rectangular plots will be delineated, marked with rebar and revegetated with

nine plantings/m^2. Broome et al. (1986) discovered that Spartina alterniflora planted 45-60 cm apart were more successful than if spaced farther apart.

Ground Water Flushing

In terms of the hydrological component of the project to be undertaken at the Bay of Isles test site, determine the rate at which water will infiltrate the soil just above the wetland. This can be done by using falling-head or constant-head permeameters Novitzki 1976). Next, estimate the rate of flow in the nearby stream at the time of the field visit, and compare that to a nearby long-term streamflow record in order to estimate the approximate low flow expected in the stream during the period of the study (Novitzki 1979). Use these data to determine the amount of water available from the stream for creating recharge and the size of impound area necessary to allow the water to infiltrate. Use pipe or flexible hose to divert water from a nearby stream, at an elevation five to ten feet above the mean high water shore line of the wetland, and transport the water to a small, shallow impoundment at the edge of the wetland.

The stream end of the pipe will be anchored at a protected location in a pool, preferably just upstream of a rock riffle. The intake point should be five to ten feet above the elevation of the edge of the wetland to allow water to flow by gravity to the recharge site. The inlet will be protected by a screen or grate to allow a reasonable intake of water for extended periods without maintenance. The pipe or hose will be anchored along its length or buried slightly below grade if possible for protection. No effort will be made to protect the system from freezing because increasing recharge (and consequently discharge) during the warm months will be adequate to demonstrate the effectiveness of this technique.

The recharge area will be created by shoveling loose soil and gravel to make a berm 12 to 18 inches high. The soil will be shoveled from the uphill side so that the soil removal area and berm together form a shallow basin. The basin should be at least 25 but no more than 100 feet long, and from 5 to 10 feet wide. The location of the outlet end of the pipe will be moved up and down hill (at the edge of the recharge area) until the flow rate approximates the desired recharge rate. The flow will be measured volumetrically, using a calibrated container and stop watch.

Project Schedule

Approximately 15 days work will be required at the Bay of

Isles* and Tonsina Bay for initial site delineation, characterization, and planting in the spring of 1991, with 5 days of follow-up monitoring in the early fall of 1991. This estimate is based on the effort of a 5-person field crew (eight to ten hour workdays) for the initial planting and a 2- person crew for the monitoring phases. If all plots exhibit plant survival, future activities will be limited to monitoring restoration success and will require 5 days of field work in the early spring and 5 days in the fall for approximately 4 years following installation. Additional time will be required to collect material and replant any plots on which no plants survived. Care will be taken not to injure sites with equipment or foot traffic. Restoration activities conducted under this project will not interfere with ongoing projects in Prince William Sound and the Gulf of Alaska.

Identification of Donor Site

Field observations in the summer of 1990 have identified several potential donor sites (transplant sources for restoration) for the Bay of Isles and Tonsina Bay:

1) Tidal marsh at the head of Outside Bay on Naked Island,
2) Tidal marsh on Crafton Island,
3) East Bay tidal marsh on Perry Island,
4) Culross Passage on Culross Island,
5) Tonsina Bay, and
6) Fringe tidal marsh around the Bay of Isles and Marsh Bay on Knight Island.

Although these sites may have potential as donor sites, they have not been investigated in detail. Therefore, the following information will be collected and used as criteria to evaluate potential donor sites:

- Species present - The composition of a tidal marsh will factor into its potential to serve as a donor site, based on the species requiring replacement at the Bay of Isles and Tonsina Bay. The site must also have an abundant supply of the appropriate species for revegetation of the Bay of Isles and Tonsina Bay.

- Oil impact - A donor site must be an "unstressed" system (void of unnatural perturbations outside of natural stress), and therefore lacking in any apparent impact from oil.

- Historical treatment record - Again, since a donor site must be "unstressed" relative to the Bay of Isles and Tonsina Bay, a potential donor must not have been

* Dependent on obtaining permission from adjacent land owner.

subjected to any type of treatment or cleanup operations.

- Vigor - To qualify as a donor site, a tidal marsh must exhibit nearly 100% cover of healthy vegetation, again demonstrating the importance of an "unstressed" system.

- Proximity of vegetative donor site - It is cost-effective and ecologically prudent to choose a donor site in close proximity to the Bay of Isles and Tonsina Bay.
- Size of donor plot - The donor site must be large enough that collection of plants for transplantation will not adversely affect the donor. Less than 1 percent cover will be removed from each donor site.

Revegetation

Plants will be installed within 48 hours after being collected. Several alternatives are available for revegetation, including: seeding, bare root, and plugs. Revegetative techniques for Carex involve collecting bare root plants from donor sites, bundling them in groups of 3, and replanting as soon after collection as is feasible. For Puccinellia, it has been shown that plugs survive and grow better than sprigs, so plugs will be used for site restoration (Seneca et al., 1982). When using Puccinellia transplants for restoration, it is important to sufficiently drain the plants.

All transplanted materials will be fertilized at the time of installation. According to Broome (1989) transplants usually benefit from fertilizer the first growing season. Either slow release or conventional water soluble fertilizers can be used. The most widely used fertilization method is approximately 15-30g per plant of slow release Osmocote fertilizer (14-14-14 analysis with a 3 month longevity) in the planting hole (Broome, 1989).

Fertilization was shown to greatly increase growth in Zostera marina in a study by Orth (1977). Fertilizer was massaged by hand into the sediment at the beginning of the experiment (repeated twice) and resulted in a large increase in leaf growth. Studies of tidal marshes affected by the *Amoco Cadiz* oil spill by Seneca et al. (1982) indicated fertilization (using Mag-Amp and Osmocote) was necessary for significant plant growth because cleanup operations had left large areas void of vegetative cover. Seneca et al. (1982) also observed higher cover for fertilized Puccinellia transplants. Fertilization needs are site-specific, however, and may not be necessary for establishing transplants. Broome et al. (1986) did not use fertilizers and succeeded in establishing a marsh (Spartina alterniflora) for at least 10 years. Fertilizer will be applied once at the outset of the project.

Site Restoration Activities

The following activities will be conducted for site restoration at the Bay of Isles and Tonsina Bay:

(1) A site description will be provided by completing a Tidal Marsh Restoration Data Form (see Attachment 1), including notations of the amount of oil originally estimated to be present in the area, according to the oil spill maps (i.e., light, moderate, heavy).

(2) Twenty-four 10 m^2 rectangular plots, will be placed within each marsh and a reference point will be established. All locations and dimensions will be noted on the data sheet.

(3) The species to be replaced will be listed, and the total number of required transplants calculated. The amount of fertilizer needed based on the total number of transplants required will be determined.

(4) A permanent reference point within each treatment plot and will be established and soil samples collected, including replicates (number to be statistically determined), using a random number table to choose the sample points. It will important to note the elevation. A 6.5 cm diameter piston corer will be used to place the sediment into solvent rinsed foil, the sediment will be wrapped, and stored (Burns and Teal, 1979). Labels will be placed on each sample and code with a unique I.D. number assigned. Tape over the label will ensure that the label adheres to the sample and does not smear. Samples will be put into an insulated cooler and transported to the laboratory for analyses. All sampling and analytical utensils contacting the sample will be rinsed with redistilled solvents before use (Burns and Teal, 1979).

(5) The soil samples will be analyzed for organic content, available nutrients (Ca, Mg, K, P, N as ammonium and nitrate), pH, and salinity at the Soil Science Lab at Oregon State University. Total hydrocarbon and weathered hydrocarbon fractions will be analyzed by SAIC Inc., in San Diego. It will be important to relate revegetation success (survival) to particular oil fractions present.

(6) Photodocumentation of the site will be made pre- and post-planting and recorded in a log book noting the film frame and roll number.

(7) Determine an appropriate donor site for both study sites.

(8) For transplantation of upper tidal marsh vegetation, the methods specified in Appendix 1 will be employed for bare root transplants (based on Spartina alterniflora using Broome, 1989):

Site Monitoring

The Bay of Isles and Tonsina Bay will require monitoring on an annual basis, at the end of the growing season in the fall of 1991, and the spring and fall of subsequent years. Monitoring results should be recorded on the Example Tidal Marsh Site Monitoring Data Form (see Attachment 2).

Ground Water Flushing Activities

Flow from the pipe will be measured several times during the spring field exercise, and once each subsequent visit. The size of the recharge area will be calculated from measurements made after the flow first begins and the pond size stabilizes, near the end of the spring field period, and once each subsequent visit. These two measurements will provide a calculation of the infiltration rate of the soil under prolonged recharge conditions which can be compared to infiltration rates calculated by the falling-head permeameters.

Other measurements will be those sediment characteristics which were planned to be measured at revegetation sites. If sediment characteristics improve most, and revegetation is most successful, near the recharge site, and improvement diminishes proportionally away from that site, there will be a clear demonstration that increased ground water discharge has accelerated sediment cleansing.

6.0 PROJECT QA ORGANIZATION AND RESPONSIBILITIES

A flowchart is particularly useful to show the QA organization of a project and to identify lines of project responsibility for each task or group of measures. Project QA organization is documented in Figure 1. The co-principal investigators (PIs) will be part of the 5-person field crew and will assume responsibility for carrying out the research tasks to ensure quality of the results generated. The Co-PIs will be primarily concerned with the QC aspects of the project. Key QA/QC responsibilities are:

QA Responsibilities
- o participate in the preparation of the QA project plan,
- o ensure that all project participants read and follow the QA project plan,
- o negotiate quality requirements with project officer,

- train field and analytical staff to perform and evaluate QC measurements,
- verify that QC activities are performed and data quality is determined as required in the QA project plan, and
- document QC outputs.

QC Responsibilities

- follow instrument manufacturer's specifications,
- perform and document preventive maintenance,
- maintain up-to-date laboratory notebooks,
- follow and document deviations from established procedures/methods,
- make data quality determinations based on QC data collected, and
- report all problems and corrective actions to the project officer

The project officer is ultimately responsible for the performance and coordination of a specific project. The project officer is management's principal contact regarding the research project. The project officer determines the quality criteria on the basis of intended use of the results to be generated and communicates those criteria to the research staff. Key QA/QC responsibilities are:

QA Responsibilities

- ensure the development of the QA project plan,
- ensure that SOPs are developed, review and approve SOPs,
- negotiate quality requirements with research staff,
- ensure that required corrective actions are implemented, and
- review project QC outputs

QC Responsibilities

- review field logbooks,
- arrange for performance evaluation or audit samples (when applicable),
- assist in scheduling audits, and
- report data quality problems to QA officer

The branch chief is responsible for all projects within a research area and for ensuring that all technical outputs meet the quality requirements of the Laboratory and the Agency. Key QA responsibilities include:

- review and evaluate work on QA implementation and progress,
- evaluate QA/QC costs,
- review and evaluate the quality of outputs generated by each project,
- review and evaluate audit and performance evaluation reports (when applicable, ensure that corrective actions are implemented), and

- develop and maintain QA-related communications channels.

Analytical laboratory staff will be required to read this QAPP and agree to comply with the program by completing the Agreement to Comply Form provided in Figure 2.

7.0 OBJECTIVES FOR MEASUREMENT

It is the responsibility of the project officer to define the intended use of the data and to develop, in cooperation with the data users, the DQOs appropriate to the project within the time and resource constraints of the effort. Data quality objectives are described in terms of precision, accuracy, completeness, representativeness, and comparability for all variables to be measured in this project. Development of DQOS must include the following steps:

- define with specificity the hypothesis, question, or objective to be addressed.

- establish guidelines for the types and quality of data needed to answer the hypothesis, question, or objective.

- explain in quantitative terms the possible errors that may arise during the measurement process.

The QA objectives for precision and accuracy for each measure (Table 1) are provided in Table 2. The method of assessing precision and accuracy using different types of quality control (QC) samples is indicated. Completeness is defined as "a measure of the amount of valid data actually obtained from a measurement process required to achieve a particular statistical level of confidence in the data compared to amount expected." The objective for completeness for this pilot project is 85%. The experimental design of this project described in Section 5 is intended to ensure that samples will be collected for oil fraction analysis that are representative of the population to be sampled. The plant transplant aspect of this project does not claim to be representative of all oil contaminated wetland sites because of the pilot-project nature of this study. There is no mandate for demonstrating comparability with other EPA or non-EPA programs for this project. However, it should be a general goal for all projects to collect data that is comparable to other data collected in this scientific field.

8.0 SAMPLING PROCEDURES

The sampling procedures used for all measurements in this project are presented in Table 3 and Appendix 1. The discussion of how sampling locations will be chosen, collection of representative samples, and sample labelling have been provided in Section 5 of this document. Table 3 provides the requirements for sample containers; sample preservation, handling and storage; and recommended holding time limits.

9.0 SAMPLE CUSTODY

Legal sample custody as required by the National Enforcement Investigations Center (NEIC) (US EPA, 1985) is not necessary for this pilot, research activity. Sample transport and handling requirements are provided in Table 3. The laboratory analyzing soil samples is located at Oregon State University, Soil Science Lab in Corvallis, Oregon. Sample collection and labelling will be documented in a field sampling logbook and a daily inventory list of all samples collected will be compiled and checked against the samples at the end of each day. Sample labels will contain site locations, data of collection, name or initials of sample collector and the type of sample (sediment, soil) will be identified. Samples will be shipped to the two analytical laboratories with an inventory list. Verification of sample receipt and evaluation of sample condition upon receipt will be documented by the analytical laboratory. Samples will be stored securely within the analytical laboratory's sample storage area at 40C. Remaining sample will be archived until analyses are completed and results are verified and validated in a secure location, clearly labelled and easily retrievable. The laboratory will track the date of sample analysis and verify that samples were analyzed within recommended holding time limits specified in Table 3.

10. CALIBRATION PROCEDURES AND FREQUENCY

When observational measures are made by more than one person, it is important to address comparability between or among observers. Calibration will include training observers by reviewing the criteria for visual observation and assessment of the condition of transplants after planting to assess viability and in evaluating viability over time. Remeasurement by all observers of 10% of the total quadrants or plots measured will be used to calibrate visual observations and provide a numerical index of variability among observers.

For analytical variables (elemental analyses) the number of standards used, their composition, and concentration will be documented by the analytical laboratory. The sample pattern will be documented to ensure that all QC samples are analyzed as required. Either high and low concentration QC check samples or certified reference standards will be used to ensure calibration

accuracy during batch sample analysis. Low concentration check samples or certified reference materials will be used to verify batch-to-batch detection limits and as an indirect method to monitor daily detection limits. The results from the analysis of at least 7 low concentration check samples are used to calculate a standard deviation. The method detection limit is the Student's T value for a one-tailed test at the 99% confidence level with n-1 degrees of freedom. It will be necessary to identify quality control check samples (QCCSs) that were used to indicate the need for recalibration as a required corrective action.

Balances used in this project will be calibrated annually under a service contract with a competent firm specializing in balance calibration and maintenance. Annual calibration will be verified by a sticker attached directly to the balance.

Project pH meters will be calibrated before use using two calibration standards bracketing the normal operating range. The calibration will be verified using a quality control check sample. Meter calibration should also be verified at the end of the analysis period.

11. ANALYTICAL PROCEDURES

Table 1 lists the methods to be used in this study. Standard and published methods are provided whenever possible. Methods for the determination of hydrocarbon fractions and weathering to be performed by SAIC are provided in Appendix 2. Methods for analyses to be performed by Oregon State University's Soil Testing Laboratory are provided in Appendix 3.

12. DATA REDUCTION, VALIDATION, AND REPORTING

Sample collection from the field can be traced by entries in the field sampling logbook, the inventory list, and the sample receipt log. Results from the analytical laboratory will be documented in both hard copy and database format on floppy disk. Raw data sheets (specifying reporting units) will be retained by the analytical laboratory. The data format required for computer file entry should be provided to the analytical laboratory. The analytical laboratory is expected to verify data entry accuracy (by visual or electronic checking procedures) of 100% of the entries. Summary statistics such as range and reasonableness checks will be used to identify outlier and error values. Data files will be backed-up regularly. Statistical tests used in final data reports will be clearly identified.

13. INTERNAL QUALITY CONTROL CHECKS

Internal QC activities ensure the quality of the data collected by verifying the precision and accuracy of analytical results in comparison to the data quality objectives specified in

Table 2. Internal QC checks also ensure that instruments are operating properly and the calibration curves are valid as sample analysis proceeds. The frequency of performing QC activities was not decided for all analytical project cooperators at the time this plan was prepared. The required frequency of the QC activities specified in Table 2 and defining the appropriate warning and control limits, and the associated corrective actions required when control limits are exceeded will be part of the contractual agreements with the participating analytical laboratories.

14. PERFORMANCE AND SYSTEM AUDITS

The QA staff of the Environmental Research Laboratory-Corvallis perform a technical systems audit (TSA) or data quality audit (DQA) of all projects. TSAs are conducted prior to or concurrent with initial data collection activities to:

- o familiarize project staff with EPA QA requirements and procedures,
- o evaluate the implementation of the QA activities specified in the QA project plan (QAPP), and
- o provide assistance in attaining the objective to collect data of known and documented quality.

Long-term projects are audited every two years or at the request of the project officer. A data quality audit (DQA) is an evaluation of the documentation associated with data quality indicators of measurement data to verify that the data are of known quality. The primary purpose of this type of audit is to verify the availability of quantitative and qualitative indicators of data quality. Availability of data quality indicators depends upon the proper collection, interpretation, and reporting of information required to characterize the quality of the data.

This project is considered a pilot study. During the first year of the project no TSAs will be conducted by ERL-C QA staff. Instead a DQA will be conducted at the conclusion of data collection activities to ensure that data meet project DQOs. However, Region 10 QA staff may elect to review either field or analytical laboratory activities.

The analytical laboratory at OSU in the Department of Soil Science will determine the organic content, nutrients, pH and salinity in soil/sediment samples. The laboratory will be required to follow this QA plan and provide the QC data specified in Table 2. This laboratory has a QA program in place which is described in Appendix 3B.

The routine QA activities practiced by SAIC are included in appendix 2.

A performance audit (PA) is a quantitative evaluation of a measurement system involving a challenge to the system by the use

of reference samples of known composition and concentration. PAs are used to determine whether a measurement system is operating

within established control limits at the time of the audit. This provides an objective assessment, in terms of precision and accuracy, of the data being generated by the system. These data may be compared to control limits established for the system (or DQOs) to identify out-of-control conditions. The results of the audit also are used to verify the accuracy and precision of data being generated in routine QC analyses conducted for the measurement system. Availability of appropriate standard or certified reference materials for the analysis of oil fractions will be investigated. This material will be analyzed in replicate in every sample batch to evaluate precision and accuracy both within and between batches.

15. PREVENTIVE MAINTENANCE

Routine preventive maintenance of all field equipment analytical instruments listed in Table 1 will be performed when QC checks indicate the need for maintenance or when dictated by routine maintenance schedules.

16. SPECIFIC ROUTINE PROCEDURES USED TO ASSESS DATA PRECISION,

ACCURACY, AND COMPLETENESS

Precision and accuracy are evaluated using the approaches specified in Table 2. **Precision** is defined as a measure of scatter among independent repeated observations or measures of the same property under prescribed conditions. Precision is usually expressed in terms of the standard deviation as:

$$s = \left(\sum_{i=1}^{n} (X_i - \bar{X})^2 / (n-1) \right)^{1/2}$$

where $\bar{X}$ is the mean of n measurements and X_i is the value of the ith measurement. Accuracy is defined as the degree to which a measured value agrees with a "true" or accepted value (or a calculated mean or median). Measures of precision and accuracy are to be completed when QC samples are analyzed and will be summarized and submitted with final data reports. Control charts will be encouraged to be used to routinely monitor precision and accuracy in the participating analytical laboratories.

17. CORRECTIVE ACTIONS

Corrective actions are performed when QC check samples indicate analytical problems, audits identify concerns, or when routine preventive maintenance indicates a problem. Table 6 provides an example of corrective actions required for an atomic absorption spectrophotomer when precision and accuracy goals are

not attained. All required corrective actions will be implemented as soon as a problem is identified. These actions will be documented and provided to the EPA project officer and Co-PIs. If required corrective actions affect data quality, the specific affected samples, observations, or other data should be explicitly identified. Caveats limiting the use of these data may be necessary when reporting final project results. All participating analytical laboratories will develop corrective action logbooks. Corrective actions required in the field can be documented in the project field notebook or on data sheets.

18. QUALITY ASSURANCE REPORTS (TO MANAGEMENT)

As discussed in Section 16, measures of precision and accuracy are to be completed when QC samples are analyzed and will be summarized and submitted with final data reports. Control charts will be encouraged to be used to routinely monitor precision and accuracy in the participating analytical laboratories. When corrective actions are required, the action taken and the results of the action can be discussed and documented in the final data report along with any problems that may affect the quality of the data or limit the use of the data.

Project deliverables and a schedule for their completion should be agreed upon between the principal investigators, the EPA project officer and all project participants. Turn-around times for analysis of soil samples and receipt of laboratory results should be clearly stated in any formal or informal agreements.

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Table 1. Project Activities and Approach

Activity	Equipment/ Approach	Method/ Reference
Donor Site Activities	plastic bags shovel metal coat hangers shovels twist-tie fasteners construction paper containers	Upper Tidal Marsh (Broome, 1989) Subtidal Marsh (Fonseca et al., 1982) and (Fonseca, 1989)
Revegetation	coring device dibble dive knife fertilizer metal coat hangers shovels twist-tie fasteners	
Site Restoration		
site description biomass percent cover vigor	Tidal Marsh Restoration Data Form (Attachment 1)	(Simensted et al. 1989)
estimate of oil spill damage	Oil Spill Maps	
plot establishment	measuring tape (m),	
soil/sediment sample	piston corer, compass	
salinity	conductivity meter	
site photodocumentation pre-planting post-planting	camera, film, logbook	
Annual monitoring visual assessment of survival and vigor	Tidal Marsh Site Monitoring Data Form (Attachment 2)	
For Puccinellia (eelgrass): density, number of shoots/ unit area	Quadrat method	(Simensted et al. 1989)

Table 1. Project Activities and Approach
(continued)

Activity	Equipment/ Approach	Method/ Reference
SOIL/SEDIMENT ANALYSIS		
SAIC, San Diego, CA		Appendix 2
hydrocarbon fraction	Methylene chloride extraction, Fluorsil column clean-up, hexane partitioning, evaporate to dryness, gravimetric analysis	
Hydrocarbon fraction weathering	capillary guard column	
Oregon State University		Appendix 3
nutrients		
Ca, Mg, K	1 N ammonium acetate extraction, atomic absorption spectrophotometer (Perkin-Elmer 372)	
phosphorus	Bray's solution extraction (if pH <7.0), molybdate blue method w/ in-line dialyzer, continuous flow analyzer, (Alpkem) (if pH> 7.0 extract with sodium bicarbonate)	
ammonium-N	KCl extraction, indophenol blue method, continuous flow analyzer, (Alpkem)	
nitrate-N	KCl extraction, Cd reduction, continuous flow analyzer, (Alpkem)	
organic content	ground to pass 0.5 mm sieve, Walkley Black titration	
soil pH	2:1 (Water:Soil) Electrode/meter	

Table 2. Objectives for Data Quality

Activity	Data Quality Parameter Evaluated
For analysis of soil/sediment samples for nutrients:	
analysis of "control" samples from site where plants were collected	Provides a blank value, verifies site was uncontaminated
analysis of low concentration QC check sample	calibration verification, detection limit verification
analysis of high concentration QC check sample	(from replicate results)
analysis of replicate samples	Evaluates sample precision (within 10%)
analysis of 3 replicates of certified reference standard/ batch	Evaluates method accuracy (within 10%) Evaluates method precision (within 10%)
analysis of low concentration spike sample	detection limit verification,
analysis of high concentration spike sample	estimation of method % recovery
For pH:	
analysis of replicate samples	Evaluates sample precision (within 5%)
analysis of 3 replicates of certified reference standard/ batch	Evaluates method accuracy (within 5%) Evaluates method precision (within 5%)
For hydrocarbon fraction, and organic content:	
analysis of replicate samples	Evaluates sample precision (within 10-15%)

Table 3. Sample Collection, Handling, and Preservation

Sample Type	Sample Container	Preservation Method/ Storage	Minimum Sample Size	Maximum Holding Time
TRANSPLANTS				
Upper Tidal Marsh Vegetation				
Carex plants	3/plastic bag	keep cool, in shade, moist	---	overnight
Puccinellia plants	plugs	keep cool, in shade, moist	---	overnight
Subtidal Marsh Vegetation				
Zostera marina	15 shoots/ clump	anchor w/ coathanger, construction paper, twist-tie in water-filled container	---	overnight
soil/ sediment	glass or plastic, cap tightly	store and transport on ice or cool	2 l.<2mm. fraction	as soon as possible after sample reaches room temperature

ATTACHMENT 1
Tidal Marsh Restoration Data Form

EXAMPLE TIDAL MARSH RESTORATION DATA FORM

Restoration Assessment

Investigators Name _____
Date _____
Time _____
Location _____
Segment Number/ID _____

1 Site Description:

2 Extent of original oiling (Based on oil map)

_____ moderate Areal percent _____

_____ heavy

3 Extent of living/dead vegetation:

i) Apparent Cover: _____%

ii) _____% aboveground biomass

iii) Belowground biomass present beyond limit of aboveground biomass?

_____ Y _____ N

if yes, extent of total marsh is _____%; location _____

4 i) Location/tidal zone of each treatment area
Permanent reference point location (landmark) _____

Distance from reference point (yards)		Elevation	
#1T	_____	#1T	_____
#2T	_____	#2T	_____
#3T	_____	#3T	_____
#4T	_____	#4T	_____
#5T	_____	#5T	_____
#6T	_____	#6T	_____

ii) substrate type (S=Sand, SH=Shale, R=Rock/Cobble, M=Mud)

#1T	_____	#4T	_____
#2T	_____	#5T	_____
#3T	_____	#6T	_____

iii) Location/tidal zone of each control area

Distance from reference point (yards)		Elevation	
#1C	_____	#1C	_____
#2C	_____	#2C	_____
#3C	_____	#3C	_____

iv) Salinity _____‰

5 Approximate area (m^2) to be restored per species:

List: species area

_____

_____

_____

6 Number of transplants needed:
9 holes/m^2 @ 3 plants/hole

	species	number
i)	_____	
ii)	_____	
iii)	_____	
iv)	TOTAL	_____

7 Amount of fertilizer needed: _____ lbs.

# of plants (6 iv) x .066 lbs. = lbs. of fertilizer needed

8 Vegetative Donor Site:

i) Proximity to restoration site (approximate miles)_____

ii) Size (m^2) _____

iii) Donor site identification number _____

9 Comments:

10 Soil Analyses (record shipping information on reverse side):

Soil sample taken? _____ Y _____ N

i) If yes, number of samples (including duplicates) _____

ii) I.D. numbers _____

iii) Method of storage _____

11 Oil characteristics at the site:

i) surface _____

ii) subsurface _____

iii) asphalt _____

iv) sheen _____

ATTACHMENT 2
Tidal Marsh Site Monitoring Data Form

EXAMPLE TIDAL MARSH SITE MONITORING DATA FORM

Investigators Name _____
Date _____
Time _____
Location _____
Segment Number/ID _____

1 Restoration method used:
i) _____ fertilization

ii) _____ transplant/fertilize

iii) Date treated _____

2 Species used for each treatment plot:

List:

#1T _____ #4T _____
#2T _____ #5T _____
#3T _____ #6T _____

3 Living/dead vegetation cover per treated and control areas:

#1T _____%
#2T _____%
#3T _____%
#4T _____%
#5T _____%
#6T _____%

#1C _____%
#2C _____%
#3C _____%

4 Substrate samples collected for oil/nutrient analysis (Y or N)

	Oil	Nutrient
#1T		
#2T		
#3T		
#4T		
#5T		
#6T		

5 Apparent vigor

	1T	2T	3T	4T	5T	6T	1C	2C	3C
i) Vigorous[1] (%)									
ii) Healthy[2] (%)									
iii) Low[3] (%)									
iv) Poor[4] (%)									
v) Dying[5] (%)									

6 Comments:

Key: [1] (healthy color, >80% cover)

[2] (healthy color, 20-80% cover)

[3] (healthy color, <20% cover)

[4] (unhealthy color)

[5] (unhealthy color - brown stems; sparse cover)

Appendix 1

Vegetation Transplant

Appendix 1

Vegetation Transplant

For transplantation of upper tidal marsh vegetation, the methods specified below will be employed for bare root transplants (based on Spartina alterniflora using Broome, 1989):

1. Obtain bare root transplants (Carex) from the edges of the selected donor tidal marshes. Loosen the plants with a shovel and remove from the marsh. Carefully remove sediment from the roots and bundle in groups of three. Using a plastic bag, place transplants in the bag so the roots are covered, and keep the roots moist.

2. To hand plant, work in pairs. The first worker creates a hole with a dibble approximately 15 cm deep, and adds 0.21 lb of fertilizer for one bundle per hole. A second worker inserts plants and firms the soil around the plants. For this project, whether there is a need to plant in pairs or individually should be determined in the field.

3. For transplantation of upper tidal marsh vegetation the following methods will be employed for plug transplants:

3a. Obtain plug transplants (Puccinellia) from a donor site by inserting a coring device approximately 20 cm into the substrate, and removing the intact plug from the ground.

3b. Remove plug from the coring device and place in plastic bags to keep the plug moist during transport.

3c. To hand plant, create a hole with a dibble or coring device large enough to hold the plug, insert 0.21 lb of fertilizer into the hole, and insert the plug. Firm the soil around the plug to anchor it.

4. Take a second picture of the site once the transplants have been planted, and mark in a log book the film frame and roll number.

5. Observations involving biomass, percent cover, and vigor will rely on the experience and professional judgement of the investigator.

Appendix 2

Methods from SAIC for Hydrocarbon Fraction and Weathering Analysis

I. SUMMARY OF METHOD OF SATURATED HYDROCARBON (HC) AND POLYNUCLEAR AROMATIC (PAH) COMPOUNDS ANALYSIS FOR SEAWATER SAMPLES

A. Sample Preservation

Methylene chloride is added to the sample in a volume ratio of 1:10 methylene chloride:seawater.

B. Sample Extraction

The extraction procedure for seawater samples is the application of EPA SW 846 Method 3510 (separatory funnel method).

B.1 Transfer the preserved known volume of seawater sample (10 ml for a shaker experiment and 100 ml for respirometric experiment) into a 250 ml separatory funnel.

B.2 Add 1 Ml of 50 ppm HC surrogate standards (o-Terphenyl or n-Decylcyclohexane) and 1 ml of 1 ppm PAH surrogate standards (Naphthalene-c8, Acenaphthene-d10, and Chrysene-d12).

B.3 Rinse the sample bottle with 20 ml methylene chloride and add the extract to the separatory funnel.

B.4 Seal and shake the separatory funnel for 1-2 minutes, with periodic venting to release excess pressure.

B.5 Allow the organic layer to separate from the water phase and collect the methylene chloride extract.

B.6 Repeat the extraction two more times using fresh portions of methylene chloride (30 ml each). The three extracts are passed through an anhydrous sodium sulfate column, and combined in a Kuderna-Denish evaporation concentrator.

B.7 The extract is concentrated to a final volume of 1 ml on the K-D apparatus.

B.8 The extract is now ready for fractionation.

C. Sample Fractionation

C.1 Activate 60/200 -mesh silica gel at 210 C for 24 hours. Prepare a slurry of 8-10 gm of activated silica gel in hexane.

C.2 Place the silica gel slurry into a 10 mm ID x 25 cm long column.

C.3 Tap the column to settle the silica gel and elute the hexane. Add 1 to 2 cm of anhydrous sodium sulfate to the top of the silica gel.

C.4 Drain the column until the solvent is just above the sodium sulfate layer.

C.5 Transfer 1 ml of the sample extract from B.8 onto the column. Just prior to exposure of sodium sulfate to the air, elute the fraction according to the following order

of solvents.

Fraction	Elution amount (ml)	Compound Class
Hexane	15 - 30	Aliphatic hydrocarbon
Hexane:Benzene	45	Aromatic hydrocarbon (1:1)

C.5 Concentrate each fraction into 1 ml using a K-D evaporative concentrator.

D. Aliphatic Hydrocarbon Analysis by GC/FID

D.1 Instrument: Hewlett-Packard 5880A Gas Chromatograph with Flame Ionization detector.

Column: 0.75 mm ID x 30 m long DB-5 with direct injection in the spitless mode.

Operating Parameter:

- Injector port temperature	250° C
- Detector temperature	350° C
- Temperature program	
initial temperature	50° C
hold time	5 minute
program rate	7° C/min
final temperature	300° C
final hold time	35 minute
final run time	75 minute or less
- Injection volume	2 uL
- Carrier gas	He 5 ml/min
- Make up gas	He 20 ml/min
- Detector	Air 240 ml/min

D.2 Calibration Curve

Prepare a five point calibration curve (Table 1) of concentration 10, 50, 100, 200, and 250 ppm in methylene chloride. Each of standard is made up of pristane, phytane, C7 through C25 and C26, C28, C30, C32, C34, C36 and C38. Add to each standard 50 ug of o-terpheyl surrogate or n-decylcyclohexane surrogate standard and 50 ug x-androstane internal standard per 1 ml of the calibration standard.

D.3 Sample Analysis

Spike 50 ug of x-androstane internal standard into each 1 ml of sample prior to analysis.

II. SUMMARY OF QA/QC PLAN

A. Aliphatic Hydrocarbon QA/QC Protocol

A.1 Relative standard deviation of response factors of five point calibration must be within ± 25%.

A.2 Daily check standard of 100 ppm is determined each day or for every 10 samples analyzed. The % difference of the response factors must be within ± 25%.

A.3 Percent recovery for n-decylcyclohexane surrogate should be within 60 - 140%.

A.4 Matrix spike and matrix spike duplicate are analyzed for every 20 samples or with every sample sent - whichever is more frequent. The spike criteria are as follows:

A.4.1 Matrix spike percent recovery of C15, C20, and C28 must be within 60 - 140%.

A.4.2 Only one compound can be below its required minimum percent recovery.

B. Aromatic Hydrocarbon QA/QC Protocol

B.1 GC/MS is tuned to meet PFTBA tuning criteria as shown below for every 12 hours of analysis.

PFTBA tuning criteria:

Mass ion	% Acceptance relative to base peak m/z 69
51	1 - 6
69 (base peak)	100
131	30 - 50
219	30 - 60
414	1 - 2

B.2 Relative standard deviation of response factors of five point calibration standard must be within 40%.

B.3 Daily check standard is determined every 12 hours of analysis. The % difference of the response factors must be within 35%.

1. MEASUREMENTS FOR OIL CHEMISTRY IN SEDIMENT AND WATER SAMPLES

Routine measurements in sediment samples are made for three variables related to oil chemistry: (1) total residue weight extracted by methylene chloride, (2) total hexane-extractable and non-hexane-extractable residue weights, and (3) hydrocarbon composition and content determined by flame ionization detector-gas chromatography (FID-GC). A flow-diagram of the protocol for analyses of these variables is shown in Figure 1. An example of the information data sheet used by laboratory personnel to record sample extraction information for sediment samples is shown in Figure 2.

Measurements in water samples are made for two variables related to oil chemistry: (1) total residue weight extracted by hexane and (2) hydrocarbon composition and content determined by flame ionization detector-gas chromatography (FID-GC). A flow-diagram of the protocol for analyses of these variables in water samples is shown in Figure 3. An example of the information data sheet used by laboratory personnel to record sample extraction information for water samples is shown in Figure 4.

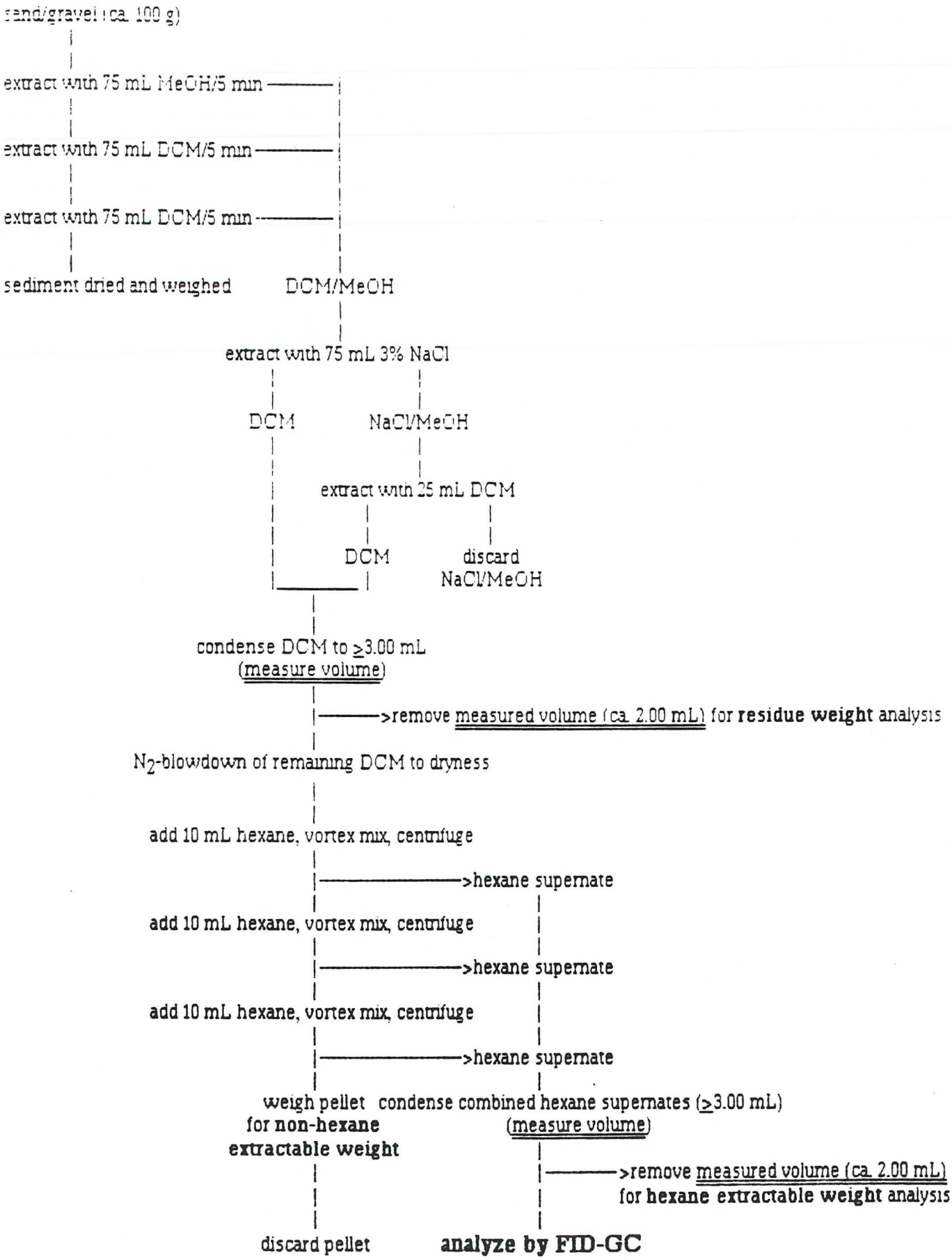
1.1. Total Residue Weight

1.1.1 Sediment samples: methylene chloride extraction/residue weight measurement

Approximately 100 g of a sediment sample in a sand/gravel size range (ca. 4-13 mm diameter) are placed in a clean 250 mL glass Erlenmeyer flask. A volume of 75 mL of pesticide-quality methanol (MeOH) is added and the flask is shaken on a mechanical shaker table for 5 min. The MeOH is then decanted through a paper filter (VWR brand, Grade No. 613) into a 500 mL separatory funnel. The sediment sample is extracted (i.e., shaken for 5 min) with two additional 75 mL volumes of pesticide-quality methylene chloride (DCM). The DCM supernates are also passed through the filter paper into the separatory funnel containing the initial MeOH extract. The weight of the sediment extracted is determined by drying the solvent-extracted sediment in an oven at 45°C, transferring the sediment to a tared weighing pan, and determining the sample weight with a Mettler Model PE160 balance capable of reading to 0.001 g.

A 75 mL volume of a 3% NaCl:freshwater solution (w:w; pre-extracted with DCM) is added to the separatory funnel containing the combined DCM-MeOH extract from the sediment sample. The funnel is then shaken for approximately 1 min, the solvent phases allowed to separate, and the DCM layer transferred to a 1000 mL glass round-bottom flask. The residual water/NaCl/MeOH solution in the separatory funnel is back-extracted (i.e., shaken for ca. 1 min) with 25 mL of DCM. Following solvent phase separation, the latter DCM layer is also transferred to the round-bottom flask. Several Teflon boiling chips are added and a three-ball Snyder column attached to the round-bottom flask. The DCM in the round-bottom is reduced in volume to approximately 5 mL over a 45°C water bath. The condensed DCM is transferred quantitatively (i.e., with DCM rinses) to a graduated cylinder. The volume in the cylinder is adjusted to a measured volume $\geq$3.00 mL under a stream of high purity nitrogen (N_2) gas. A measured aliquot

SAND/GRAVEL SAMPLE EXTRACTION (ca. 100 g extracted)



EPA/SAIC 1990 Bioremediation Program
Extraction Sheet for Oil Analysis—Sediment Sample
DCM\Hexane-Extractable Procedure

I. Sample Identification:
SAIC Analysis Identification Number: _____
Batch Identification Number: _____
Sample Identification Number: _____
Miscellaneous Sample Information:

II. **Sample Extraction/Residue Weight Determination:**
a) Extraction Analyst _____ Date of Extraction: _____
b) Extraction Information:
i) Dry Weight of Sediment Extracted: _____ g (1)
ii) Solvent Extractions:
initial MeOH/DCM extractions:
volume MeOH/agitation time: _____
volume DCM/agitation time: _____
number of DCM extractions: _____
back-extraction:
volume of 3% NaCl/agitation time: _____
volume DCM/agitation time: _____
number of DCM extractions: _____
iii) Final DCM Extract
Final extract volume [A]: _____ mL (2)
Residue weight determination for final extract
volume for residue weight measurement [B]: _____ mL (3)
residue weight measurements:
tare: _____ g
residue + tare measurement #1: _____ g
residue + tare measurement #2: _____ g
measured DCM residue weight [C] _____ g (4)
total residue weight in final sample extract [C x (A/B)]: _____ g
Residue weight/dry weight of sediment g/g

III. **Hexane-Extractable/Non-Hexane-Extractable Weight Determinations:**
a) Extraction Analyst _____ Date of Extraction: _____
b) volume of final DCM extract used for hexane-extraction step [D]: _____ mL (5)
c) hexane extractions:
i) volume hexane/agitation time: _____
number of hexane extractions: _____
ii) final hexane extract
final volume [E]: _____ mL (6)
hexane-extractable and non-hexane-extractable weight determinations:
vol. of fin. extr. taken for hexane-extractable weight measurement [F]: _____ mL (7)
weight measurements:

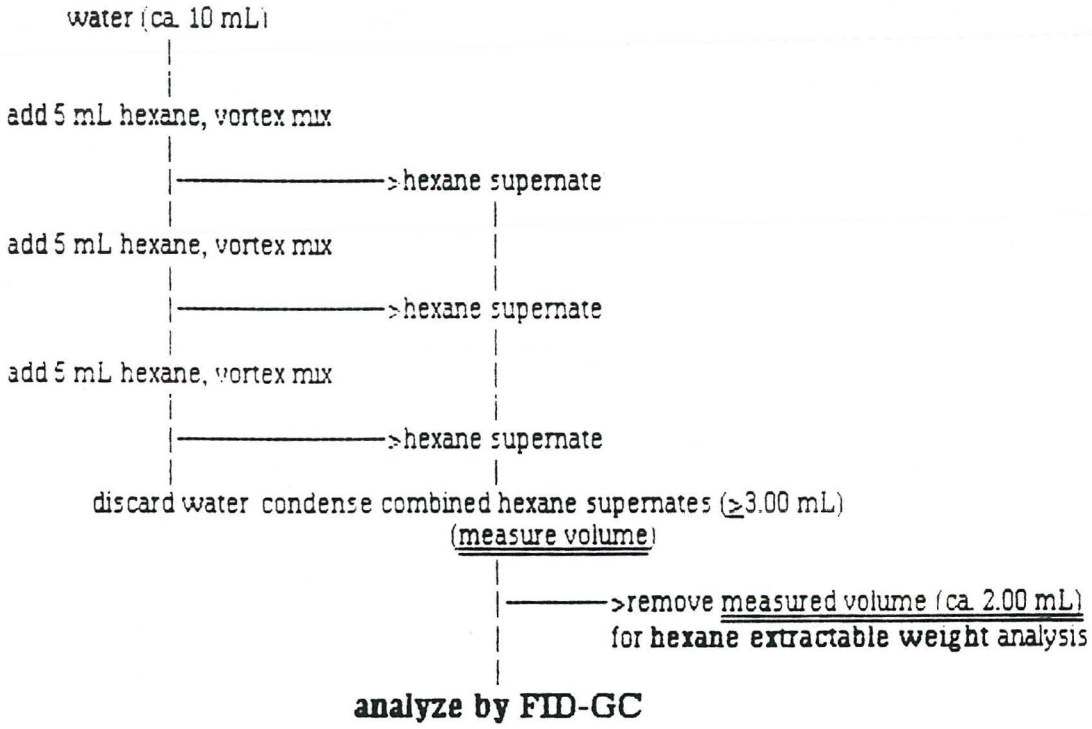
	hex-extractable	non-hex-extractable	
tare:	_____ g	_____ g	
residue + tare measurement #1:	_____ g	_____ g	
residue + tare measurement #2:	_____ g	_____ g	
measured weights [G]	_____ g	_____ g	(8)
total weights in sample:	{Gx(E/F)x(A/D)}= _____ g	{Gx(A/D)=} _____ g	

Hexane-extractable weight/total residue weight of sample: _____ %
Non-hexane-extractable weight/total residue weight of sample: _____ %
Hexane-extractable/Non-hexane-extractable ratio:

IV. **GC Sample Run Information for Hexane-Extractable Fraction:**
a) Date GC vial crimped: _____ GC operator: _____
c) HP instrument ID: _____ PIV (uL): _____
d) HP run date: _____ HP run number: _____
g) GC file ID for sample: Dilution factor:

V. **General Comments:**

WATER SAMPLE EXTRACTION (ca. 10 mL extracted)



EPA/SAIC 1990 Bioremediation Program
Extraction Sheet for Oil Analysis—Water Sample
(hexane extraction method)

I. Sample Identification:
SAIC Sample #: _____
Batch ID: _____
Sample Number: _____
Collection Site: _____
Collection Date: _____
Miscellaneous Sample Information: _____
_____

II. Sample Extraction:
a) Extraction Analyst: _____ Date of Extraction: _____
b) Extraction Information:
i) Volume of Water Extracted: _____ mL
ii) Solvent Extractions:
extraction:
volume hexane/agitation time: _____
number of hexane extractions: _____
iii) Final Sample Extract:
Final extract volume [A]: _____ mL
Residue weight determination for final extract:
vol. of fin. extr. taken for res. wt. measurement [B]: _____ mL
residue weight measurements:
tare: _____ g
residue + tare measurement #1: _____ g
residue + tare measurement #2: _____ g
residue + tare measurement #3: _____ g
measured residue weight [C] _____ g
total residue weight in final sample extract [C*(A/B)]: _____ g
Residue weight/volume of water: _____ mg/L

III. GC Sample Preparation/Run Information:
a) Date vial crimped: _____ GC operator: _____
c) HP instrument ID: _____ IV/PIV (uL/uL): _____
d) HP run date: _____ HP run number: _____
g) GC file ID for sample: _____
h) Comments: _____
_____

(ca. 2.00 mL) is transferred to a tared aluminum weighing pan and allowed to dry at room temperature to a constant weight. Total residue weight for the sample is determined as the difference between the weight measurements for the weighing pan+sample and the pan tare, with appropriate correction being made for the volume fraction of the initial DCM extract used for the weight measurement. Weight measurements for the pan tare and sample+tare are made with a Mettler Model PE100 balance capable of reading to 0.0001 g. The residual DCM fraction of the sample not used for the total residue weight measurement is used for the following hexane-extraction procedure (Section 1.2).

1.1.1 Water samples: hexane extraction/residue weight measurement

A water sample having a 10.0 mL volume is contained in a 20-mL VOA vial equipped with a Teflon-lined screw cap. A 5 mL volume of pesticide-quality hexane is added and the vial is mixed on a Vortex Mixer for ca. 1 min. Following phase separation, the hexane is transferred to a graduated cylinder with either a gas-tight syringe or a disposable Pasteur pipet. This extraction procedure is repeated with two additional 5-mL volumes of hexane, which are also transferred to the graduated cylinder. The hexane volume in the cylinder is adjusted to a measured volume $\geq$3.00 mL under a stream of high purity nitrogen (N_2) gas. A measured aliquot (ca. 2.00 mL) is transferred to a tared aluminum weighing pan and allowed to dry at room temperature to a constant weight. Total residue weight for the sample is determined as the difference between the weight measurements for the weighing pan+sample and the pan tare. Weight measurements for the pan tare and sample+tare are made with a Mettler Model PE100 balance capable of reading to 0.0001 g.

1.2. Hexane-Extractable and Non-Hexane-Extractable Weight: hexane extraction of initial DCM residue fraction—Sediment Samples Only

A measured volume of the condensed DCM extract from Section 1.1.1 is reduced to dryness in a glass tube under a stream of high purity nitrogen (N_2) gas. A 10 mL volume of pesticide-quality hexane is added to the tube and mixed with a Vortex Mixer for ca. 1 min. The tube is then centrifuged to precipitate the "non-hexane-extractable" fraction, and the "hexane-extractable" supernate is transferred to a clean glass tube. The hexane (10 mL) vortexing/centrifugation step is repeated two additional times. All hexane supernates (i.e., the "hexane-extractable" fraction) are combined in the glass tube and reduced in volume to approximately 2-4 mL under a stream of N_2 gas. The extract is then transferred quantitatively (i.e., with hexane rinses) to a 10 mL graduated cylinder and the exact volume recorded. A measured aliquot (ca. 2.00 mL) of the extract is then transferred to a tared aluminum weighing pan and allowed to dry at room temperature. Total hexane-extractable weight for the sample is determined from the extract weight in the pan, with appropriate corrections being made for the sample volume fractions used for weight measurements in this section and Section 1.1.1. Total non-hexane-extractable weight for the sample is determined by allowing the hexane-extraction tube to dry (i.e., after removal of the final hexane supernate), determining its weight, removing all of the non-hexane-extractable pellet with DCM rinses, drying the tube again, and measuring its final weight. The non-hexane-extractable weight for the sample is determined from the difference

in the tube weights with and without the non-hexane-extractable pellet, with appropriate corrections being made for the volume of the initial sample extract removed for the total residue weight measurement (Section 1.1.1). Weight measurements for the hexane-extractable and non-hexane-extractable weight determinations are made with a Mettler Model PE100 balance capable of reading to 0.0001 g.

1.3. Measurement of hydrocarbon composition and content by FID-GC analysis

Analysis of sample extracts for hydrocarbon composition and content are performed on a Hewlett-Packard (HP) 5890 gas chromatograph (GC) equipped with a flame ionization detector (FID), a HP 7673A autosampler and controller, an 82169A HP-IL/HP-IB Interface, a HP 3396A integrator, and a HP 9133H disk drive. A fused silica capillary chromatography column in the GC is used for compound separations. GC operating and temperature programming parameters used for analyses are:

GC column: DB-5 liquid phase, 30 m length X 0.32 mm ID, 0.25 um film thickness (J&W Scientific, 91 Blue Ravine Road, Folsom, CA 95630)
GC injection mode: splitless, 1 min valve closure
GC injection temperature: 285°C
GC detector temperature: 350°C
GC oven programming rate:
initial temperature: 45°C, 5 min hold
temperature ramp: 3.5°C/min
final temperature: 280°C, 20 min hold
sample injection volume: 1.0 uL

Quantitation for hydrocarbons is accomplished with an external standard method. The standard solutions consist of aliphatic hydrocarbons containing a sequential mixture of n-alkanes with even and odd numbers of carbon atoms (n-C8 through n-C30 plus n-C32) and the isoprenoid compounds pristane and phytane. Integration of peaks in all chromatograms (i.e., standards and samples) is accomplished with valley-to-valley baseline placement.

A mixture of polynuclear aromatic hydrocarbons (PAHs) consisting of compounds with two to six ring structures was also used in an attempt to identify PAH compounds in sample extracts. PAH compounds could not be reliably identified in the FID-GC chromatograms of sample extracts in the absence of a physical separation of aliphatic and aromatic fractions for sample extracts. Hence, identification and quantitation of PAH compounds will require additional treatment of the sample extracts (e.g., physical separation of aliphatic and aromatic fractions and/or gas chromatography-mass spectrometry analysis).

1.3.1. Standard solutions for FID-GC analyses

Table 1

n-ALKANE STANDARD SOLUTIONS FOR FID-GC

Compound	conc in primary standard (ng/uL)	concentrations of aliphatics in working standard solutions (ng/uL) [125]	[25]	[5]
nC-8	244	122	24.4	4.88
nC-9	260	130	26.0	5.20
nC-10	248	124	24.8	4.96
nC-11	248	124	24.8	4.96
nC-12	260	130	26.0	5.20
nC-13	272	136	27.2	5.44
nC-14	260	130	26.0	5.20
nC-15	272	136	27.2	5.44
nC-16	268	134	26.8	5.36
nC-17	320	160	32.0	6.40
pristane	268	134	26.8	5.36
nC-18	248	124	24.8	4.96
phytane	256	128	25.6	5.12
nC-19	252	126	25.2	5.04
nC-20	432	216	43.2	8.64
nC-21	248	124	24.8	4.96
nC-22	276	138	27.6	5.52
nC-23	320	160	32.0	6.40
nC-24	244	122	24.4	4.88
nC-25	260	130	26.0	5.20
nC-26	252	126	25.2	5.04
nC-27	244	122	24.4	4.88
nC-28	244	122	24.4	4.88
nC-29	256	128	25.6	5.12
nC-30	240	120	24.0	4.80
nC-32	240	120	24.0	4.80

NOTE: all standards in 100% hexane

Table 2

PAH STANDARD SOLUTION FOR FID-GC

Compound	concentrations of PAHs (ng/uL)
naphthalene	66.2
2-methylnaphthalene	65.0
1-methylnaphthalene	67.5
biphenyl	66.2
2,6-dimethylnaphthalene	66.2
acenaphthylene	63.6
acenaphthene	68.6
2,3,5-trimethylnaphthalene	59.1
fluorene	65.5
phenanthrene	66.1
anthracene	50.1
1-methylphenanthrene	65.5
fluoranthene	65.9
pyrene	66.1
benz[a]anthracene	56.8
chrysene	66.1
benzo[b]fluoranthene	65.9
benzo[k]fluoranthene	65.9
benzo[e]pyrene	66.2
benzo[a]pyrene	59.6
perylene	49.8
indeno[1,2,3-c,d]pyrene	58.6
dibenz[a,h]anthracene	49.9
benzo[ghi]perylene	58.6

NOTE: standard in 100% toluene; obtained from NIST

The primary standard for aliphatic hydrocarbons is prepared by combining known quantities of neat n-alkane (n-C8 through n-C30 plus n-C32) and isoprenoid (pristane and phytane) compounds in a volumetric flask and bringing to volume with hexane. Nominal concentrations for compounds are 250 ng/uL, with specific concentrations being shown in Table 1. Working solutions of the standards are prepared at three concentration levels for FID-GC analyses by appropriate dilutions of the primary standard with hexane. Nominal concentration levels of individual compounds in the working standards are 125 ng/uL, 25 ng/uL, and 5.0 ng/uL, with specific concentrations being shown in Table 1.

The standard used in attempts to identify PAH compounds in sample extracts is a certified standard solution prepared by NIST (i.e., the National Institute of Standards and Technology, Gaithersburg, MD). Compound concentrations in this standard are approximately 60 ng/uL, with specific concentrations being shown in Table 2.

1.3.2. Initial stability calibration of the FID-GC

Before sample extracts can be analyzed for their hydrocarbon content and composition, the FID-GC must meet specified initial instrument stability calibration criteria. These calibration criteria are performed with injections of the three working aliphatic standard solutions (i.e., nominal concentrations of 5, 25, and 125 ng/uL for compounds). Peaks for compounds in chromatograms of the standard solutions must be 90% resolved. Peak resolution (PR) is calculated with the following formula:

PR = [1 - (height of valley between 2 peaks/height of smaller of 2 peaks)] x 100.

Retention times (RTs) for all identified compounds in the standard solutions must also vary by no more than $\pm$1.0% from the mean RT for the three injections. Finally, response factors (RFs) for aliphatic compounds in the working standards must meet certain reproducibility criteria related to instrument response. RFs are calculated as:

RF_X = ng of compound x on column/GC area counts for compound x.

In the three working solutions for the aliphatic standards, the RFs for nC-17, pristane, nC-18, and phytane must not vary by more than $\pm$25% from the mean value for the three solutions. No more than three of the RFs for all remaining n-alkanes can vary by >$\pm$40% from their respective means for the three standard solutions.

If an injection for one of the three standard solutions for the aliphatic standards is responsible for failure to meet the preceding criteria, the "offending" standard solution can be injected one additional time. If the result of the reanalysis meets the stability criteria, injection of sample extracts can begin. If results of the reanalysis do not meet the stability criteria, sample extracts cannot be run and routine maintenance must be performed on the instrument to correct the problem.

1.3.3. Ongoing calibration of the FID-GC

Following initial instrument calibration, analysis of sample extracts occurs. However, the FID-GC must be recalibrated (i.e., checked for ongoing stability of the instrument response) with an aliphatic standard solution at least once every 24 hours. The standard solution for this ongoing stability calibration is the 25 ng/uL working standard for the aliphatics. RFs for nC-17, pristane, nC-18, and phytane in the ongoing standard calibration check cannot vary by >30% from the means obtained in the initial 3-point calibration (Section 1.3.2.). No more than three of the RFs for all remaining n-alkanes can vary by >40% from their means in the initial 3-point calibration.

If the ongoing calibration check for the aliphatic standard does not meet the preceding instrument stability criteria, the standard solution can be injected one additional time. If the result of the reanalysis meets the stability criteria, injection of sample extracts can continue. If results of the reanalysis do not meet the criteria, a new 3-point initial stabilization procedure must be initiated (Section 1.3.2) and/or routine instrument maintenance on the GC must be performed. All sample extracts injected after the last acceptable calibration check must also be reanalyzed on the FID-GC.

1.3.4. Quantitation for concentrations of identified n-alkane and isoprenoid compounds

N-alkane and isoprenoid (i.e., pristane and phytane) compounds are quantified in FID-GC chromatograms of sample extracts by an external standard method that uses the aliphatic standard solutions used to calibrate the GC. Initial and ongoing instrument stability criteria for the FID-GC must be acceptable (i.e., Sections 1.3.2 and 1.3.3) for quantitation to proceed for sample extracts. For samples, chromatographic peaks are identified as aliphatic compounds by comparison with retention times for specific compounds in the closest preceding injection of the aliphatic standard solution. The retention time (RT) for a peak in a sample chromatogram must be within ±1.0% of the absolute RT for the compound in the standard for assignment of compound identity.

Final concentrations for hydrocarbons identified by FID-GC in sample extracts will be reported in units of mass of a hydrocarbon compound per unit mass of total residue weight (i.e., DCM-extractable residue for sediments, as determined in Section 1.1). Calculation of these hydrocarbon concentrations is done with the formula:

$$C_X = (A_X \times RF_X) \times (vol_{fin.hex.}/vol_{GC\ inj}) \times (vol_{fin.DCM}/vol_{for\ hex\ ext}) \times (1/tot.res.wt.)$$

where

C_X = concentration of analyte x per unit of total residue weight (in g/g),
A_X = FID-GC area counts for analyte x,
RF_X = response factor for analyte x (see Section 1.3.2),
$vol_{fin.hex.}$ = total volume of the final hexane-extractable fraction analyzed by FID-GC,
$vol_{GC\ inj}$ = volume of the hexane-extractable fraction injected into the FID-GC,

$vol_{fin.DCM}$ = final volume of the initial DCM extract,
$vol_{for\ hex\ ext}$ = volume of initial DCM extract processed through the hexane-extractable procedure, and
tot.res.wt. = total DCM-extractable residue weight for the sample (in g, see Section 1.1).

Values for RF_X in this calculation are obtained from the closest preceding injection of the mid-level aliphatic (i.e., 25 ng/uL) standard injected into the GC.

1.3.5. Quantitation for concentrations of total resolved peaks and the unresolved complex mixture (UCM) in FID-GC chromatograms

FID-GC chromatograms of sample extracts will normally contain (1) a variety of resolved hydrocarbon peaks including identified n-alkanes, pristane, and phytane as well as other unidentified peaks and (2) an unresolved complex mixture (UCM) that appears as a "hump" above the background chromatogram baseline and beneath the resolved peaks. Aliphatic compounds identified in the resolved peak fraction are quantified as described in Section 1.3.4.

Concentrations for unidentified resolved peaks in a sample extract between two contiguous n-alkanes (exclusive of pristane and phytane) are estimated by summing areas for all resolved peaks between the two n-alkanes and using the following formula:

$$C_y = (A_y \times RF_y) \times (vol_{fin.hex.}/vol_{GC\ inj}) \times (vol_{fin.DCM}/vol_{for\ hex\ ext}) \times (1/tot.res.wt.)$$

where

C_y = estimated concentration for all resolved peaks between two adjacent n-alkanes per unit mass of total residue weight,
A_y = FID-GC area counts for the sum of the resolved peaks between the two adjacent n-alkanes
RF_y = mean of the response factors for the two adjacent n-alkanes (see Section 1.3.2),
$vol_{fin.hex.}$ = total volume of hexane-extractable fraction that was analyzed by FID-GC,
$vol_{GC\ inj}$ = volume of hexane-extractable fraction injected into the FID-GC,
$vol_{fin.DCM}$ = final volume of initial DCM extract,
$vol_{for\ hex\ ext}$ = volume of initial DCM extract used for hexane-extractable measurement,
tot.res.wt. = total DCM-extractable residue weight for sample.

The concentration for the total resolved peaks is calculated as the sum of all identified aliphatics (i.e., n-alkanes plus pristane and phytane; Section 1.3.4) plus the sum of the concentrations for the unidentified resolved peaks between all adjacent n-alkanes as computed with the preceding equation.

Hydrocarbon concentrations for the UCM in a sample extract are estimated by determining the area of the UCM in a FID-GC chromatogram with an electronic digitizing tablet (Kurta Corporation). The estimated concentration for the UCM is calculated as:

$$C_Z = (A_Z \times P_Z \times RF_Z) \times (vol_{fin.hex.}/vol_{GC\ inj}) \times (vol_{fin.DCM}/vol_{for\ hex\ ext}) \times (1/tot.res.wt.)$$

where

C_Z = estimated concentration for the UCM per unit mass of total residue weight,

A_Z = area of the UCM "hump" in the chromatogram in digitizing tablet units (at a specified attenuation)

P_Z = factor for converting digitizing tablet area units to equivalent GC area units (determined for the attenuation specified for A_Z)

RF_Z = mean of the response factors for all n-alkanes between the nC-12 and nC-32 (see Section 1.3.2 for RF determination),

$vol_{fin.hex.}$ = total volume of hexane-extractable fraction that was analyzed by FID-GC,

$vol_{GC\ inj}$ = volume of hexane-extractable fraction injected into the FID-GC,

$vol_{fin.DCM}$ = final volume of initial DCM extract,

$vol_{for\ hex\ ext}$ = volume of initial DCM extract used for hexane-extractable measurement,

tot.res.wt. = total DCM-extractable residue weight for sample.

The P_Z factor for the digitizing tablet is determined by comparing areas for two n-alkane peaks (usually nC-15, nC-23, pristane, and/or phytane) in chromatograms for all of the sample extracts obtained by both the digitizing tablet and the GC integrator (the latter being the method used for all resolved peaks in chromatograms). The overall mean of these values from all of the sample chromatograms was used as the P_Z value. Digitizing tablet area units are dependent on the attenuation at which a chromatogram is run, whereas area counts determined by the electronic integrator attached to the GC are not. Hence, the P_Z factor for converting digitizing tablet area units to equivalent GC area units for UCM determinations is dependent on the attenuation used for a particular chromatogram.

1.4. QC (Quality Control) procedures for measurements related to oil chemistry

1.4.1. Method blanks

Analytical method blanks involve analysis of solvent blanks through the analytical procedures illustrated in Figures 1 and 3. Method blanks are analyzed with a frequency of at least 1 for every 12 field samples.

1.4.2. QCCS (Quality Control Check Samples)

A QCCS involves analysis of a known weight of unweathered Prudhoe Bay crude oil through the analytical procedures illustrated in Figures 1 and 3. QCCS samples consist of a weighed amount of unweathered Prudhoe Bay crude oil (ca. 100 mg). Data for repeated analyses of QCCS samples should remain relatively constant, and the data are used to control and assess precision and accuracy. Variables monitored in QCCS samples include (1) total residue weight (as a percentage of the initial crude oil weight used for the QCCS sample), (2) total hexane-extractable and non-hexane-extractable weights (as percentages of the initial crude oil weight), (3) concentrations of individual n-alkanes, pristane, phytane, total GC-resolved peaks, and the UCM per unit mass of the initial oil, and (4) concentration ratios for nC-17/pristane, nC-18/phytane, and total GC-resolved peaks/UCM. Control charts will be developed and monitored for these variables in QCCS samples during the course of analyses. QCCS samples will be analyzed with a frequency of at least 1 for every 12 field samples during routine oil analyses.

The ideal material for the QCCS analyses would be a naturally weathered North Slope crude oil. However, sufficient quantities of such a material are not readily available. Therefore, unweathered Prudhoe Bay crude is used for the QCCS. When using unweathered crude, it must be recognized that volatile components (e.g., lower molecular weight compounds) will be lost during certain sample treatment steps (e.g., the drying step to determine total residue weight). However, these lower molecular weight components will already be absent from oil obtained from field samples due to evaporation and dissolution processes that have previously affected the oil. Effects of evaporation losses of more volatile components on overall weights for unweathered Prudhoe Bay crude oil have been investigated, with results being illustrated in Figure 5. Data for the figure were generated by putting 280-300 mg of unweathered Prudhoe Bay crude oil in weighing pans, maintaining the pans open to the atmosphere at room temperature (70-80°C), and taking pan weights over time. Results in the figure indicate that total residue weight recoveries for QCCS samples of unweathered Prudhoe Bay crude oil should be on the order of only 75% following drying of the residue extract in a drying pan.

1.4.3. Analytical triplicates/duplicates

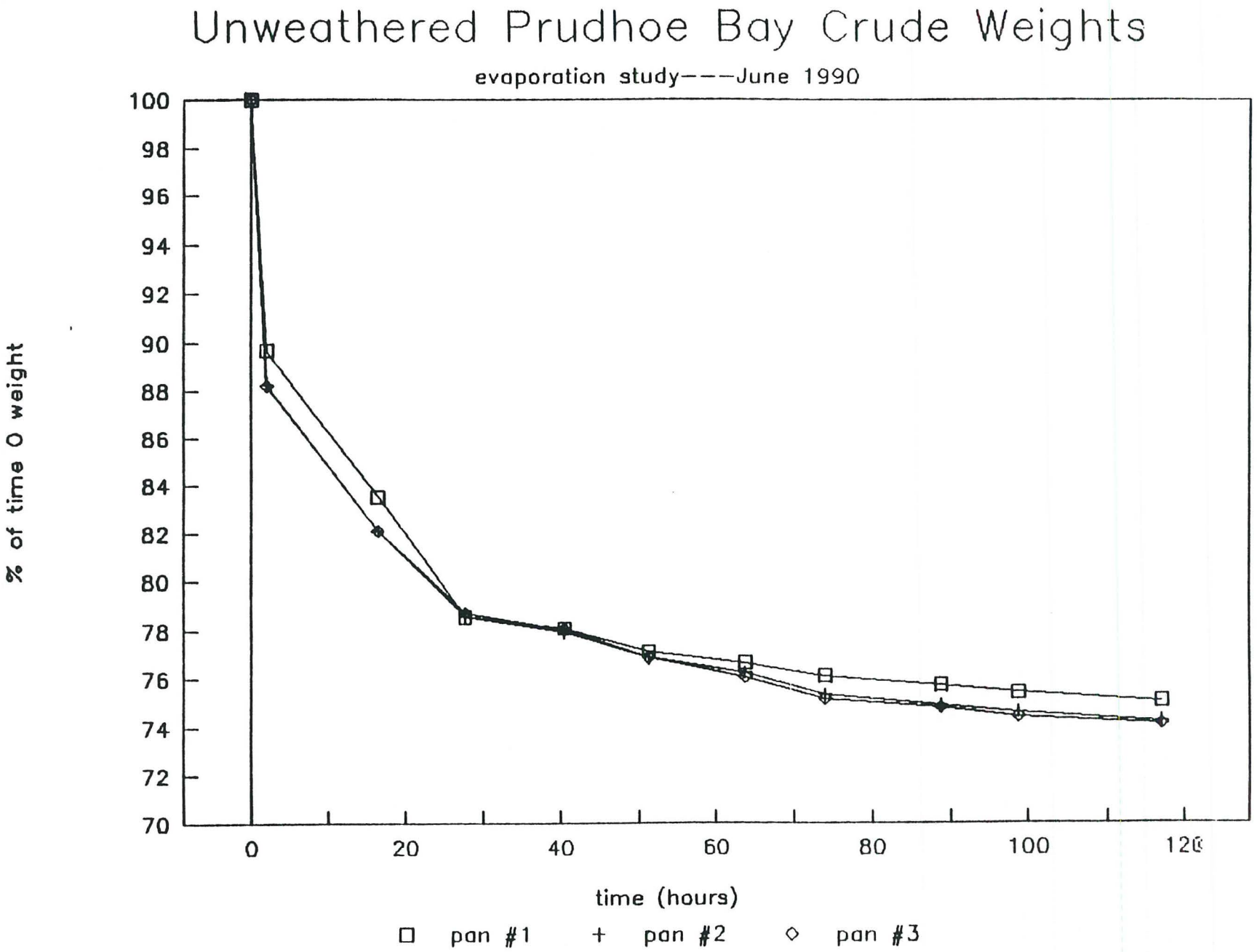
Triplicate or duplicate subsamples of selected field samples will be analyzed to evaluate "within batch" variability during laboratory analyses. Precision for these analyses are calculated as relative standard deviations (RSD) for triplicates or relative percent differences (RPDs) for duplicate analyses of samples. RSD as a percent is calculated as:

$$RSD = (\text{standard deviation/mean}) \times 100.$$

RPD as a percent is calculated as:

$$RPD = [(x_1 - x_2)/x_{ave}] \times 100$$

where x_1 and x_2 are measured values for two analyses and x_{ave} is the mean of the two analyses. The frequency of the triplicate/duplicate analyses is according to decisions made by the EPA


Unweathered Prudhoe Bay Crude Weights
evaporation study---June 1990
% of time 0 weight
100
98
96
94
92
90
88
86
84
82
80
78
76
74
72
70
0
20
40
60
80
100
120
time (hours)
pan #1
pan #2
pan #3

Principal Scientist (H. Pritchard) and/or the project data management team (e.g., D. Heggem, A. Neale, D. Chaloud).

1.4.4. QAX (Quality Control Check for Extraction Efficiency)

To estimate extraction efficiency for oil from sediment samples, a second extraction (i.e., a QAX) for selected samples will be performed using the protocols described in Sections 1.1, 1.2, and 1.3. The QAX extract will be processed in an identical manner to the initial sample extract. Results of the QAX analysis will be taken to represent complete extraction of oil from samples if values for measured variables (e.g., total residue weight, hexane-extractable and non-hexane-extractable weights, and/or FID-GC measured concentrations for hydrocarbons) in the QAX are <15% of values measured in the initial extraction of the sample.

1.5. Detection limits

1.5.1. Sediment dry weight

Dry weights for sediments extracted for oil chemistry are measured with a Mettler Model PE160 balance capable of reading to 0.001 g. Sediment samples extracted for this program will be in the range of 100 g dry weight.

1.5.2. Total (DCM-extractable) residue weights and hexane-extractable and non-hexane-extractable weights

Measurements for both the total (i.e., DCM-extractable) residue weight (Section 1.1.1) and hexane-extractable and non-hexane-extractable weights (Section 1.2) for sediment samples are measured with a Mettler Model AE100 balance capable of reading to 0.0001 g.

1.5.3. Hydrocarbon compound concentrations by FID-GC

The HP5890 FID-GC (see Section 1.3) is capable of detecting approximately 0.0001 ug (i.e., 1×10^{-10} g) of an individual n-alkane compound injected into the GC. Using the formula in Section 1.3.4 for sediment samples, this yields a detection limit of approximately 1 ug/g of total residue weight for an individual n-alkane compound in a sediment sample with the following assumptions:

(1) a final sample hexane volume of 4.00 mL,
(2) an FID-GC sample injection volume of 1.00 uL,
(3) a final DCM extraction volume of 4.00 mL,
(4) 2.00 mL of the DCM volume used for the hexane extraction procedure, and
(5) a total DCM-extractable residue weight for a sample of 0.8 g (i.e., a residue amount frequently measured in extractions of 100 g of sediment from field samples for the 1990 Bioremediation Program).

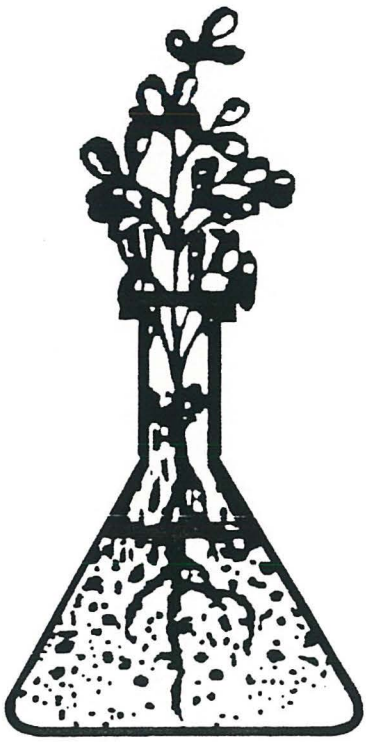
Specific detection limits for n-alkane compounds will vary between samples because items 1, 3, 4, and 5 in the preceding assumptions will vary between samples.

Appendix 3A

Oregon State University, Soil Testing Laboratory Methods

Methods of Soil Analysis Used in the Soil Testing Laboratory at Oregon State University

D. A. Horneck, J. M. Hart, K. Topper, B. Koepsell



Methods of Soil Analysis Used in the Soil Testing Laboratory at Oregon State University

D. A. Horneck, J. M. Hart, K. Topper, B. Koepsell*

INTRODUCTION

General

This manual describes and documents procedures used in the Oregon State University Soil Testing Laboratory (OSUSTL), and to supply information on the appropriate documentation of these methods. Of the numerous methods for soil analysis, research at Oregon State University indicates that the procedures outlined in this publication are suitable for Oregon conditions.

The Cascade Mountain Range is a natural boundary that separates Oregon into eastern and western sectors. Western Oregon soils tend to be acidic, while the soils in eastern Oregon tend to be slightly acidic or alkaline. In view of these differences, some testing procedures differ for eastern and western Oregon. For example, the phosphorus test for western Oregon requires a dilute acid-fluoride (Bray P1) extraction solution, while sodium bicarbonate is used for samples from eastern Oregon.

Although reference is made to specific scientific supplies and instruments used in the OSUSTL, similar equipment from other manufacturers can be substituted. Mention of a model or brand name is neither an endorsement nor a promotion for the product.

The appendix contains a combination of alternate procedures, seldom used procedures and instructions for standardization of an acid.

Future Considerations

Improving analytical procedures for fertilizer recommendation is an on-going project at the College of Agricultural Sciences, Ag. Experiment Station, Extension Service, Department of Soil Science and the OSUSTL. Consequently, after thorough research, soil testing procedures and methods of reporting are periodically updated. Comments from the farming and university communities, along with suggestions from the fertilizer industry, commercial laboratories, and agriculture consultants are considered. Future topics for research include:

1. Using a volume scoop for routine analyses versus weighing samples.
2. Evaluating a universal extractant, such as Melich III for analyses performed on an ICP.
3. Computerizing of data acquisition from laboratory equipment.

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*Donald A. Horneck, research assistant, and John Hart, Extension soil scientist, Oregon State University. Karl Topper, research assistant, Utah State University; formerly research assistant, Oregon State University. Barbara Koepsell, lab technician, Oregon State University.

4. Rewriting the computer program which prints and writes fertilizer recommendations.
5. Compile an annual report that includes data from other soil testing laboratories.

When major analytical changes are accepted, an updated edition of this publication would be made available.

Collection and Preparation of Soil Samples

Collecting soil samples from the field is an integral part of soil testing. Samples must represent the soil in the field from which it is taken. This involves obtaining 20-40 subsamples per sample submitted for analysis. Information on soil sampling is provided in Oregon State University Extension Circular 628, "How to Take a Soil Sample and Why." Sampling instructions are also available at county Extension offices or from OSUSTL.

Samples should be submitted in a standard soil sample bag or in a plastic container. Plastic containers are preferable to metal containers for collecting and mixing soil samples. Contamination may be a problem for boron (B) and zinc (Zn) when samples are collected and stored in certain kinds of paper bags. In the field, extreme care is necessary to avoid contaminating the soil sample with fertilizer or with extraneous materials from the sampling tools.

When the soil samples arrive at the OSUSTL, they are placed on trays and dried in a forced-air drying cabinet at 35 C or lower. Drying at higher temperatures may affect analytical results. Soil samples normally dry in 24 to 48 hours and are then pulverized and sieved with a Custom Laboratory Equipment Co. Dynacrush soil crusher.[1] Soil passing through the 14-mesh (2 mm) stainless steel sieve is returned to the original sample bag and stored for analysis. OSUSTL releases soil test results and fertilizer recommendations immediately after sample analysis has been completed. Soil samples are stored for future reference for 4 to 6 months, then discarded.

Accuracy and Precision

Laboratory instruments are calibrated using standard solutions that are either purchased commercially or mixed by the OSUSTL. Standard soil samples are also maintained as reference samples for evaluating

Documentation of Methods

The analytical methods used in the OSUSTL, including appropriate literature citations, are outlined in the following sections. Modifications of the published methods with respect to changes in reagents or in procedural detail is described under "Comments." Some procedures have been modified to facilitate the use of a continuous-flow analyzer. Since this equipment is not available in all laboratories, alternative procedures are also reported. A general reference for procedures used in analyzing soil is *Methods of Soil Analysis* published by the American Society of Agronomy in Madison, Wisconsin (1982).

Use of ppm

In this manual the use of parts per million (ppm) is meant to be equivalent to milligrams per liter (mg/L) or milligrams per kilogram (mg/kg), with the weight of 1 liter of water equal to 1000 g or 1 kg. General use of ppm follows:

ppm = mg/L = mg L^{-1}
for solids weighed in water

ppm = mg/kg = mg kg^{-1}
for results on a dry weight basis

ACKNOWLEDGEMENTS

The authors wish to acknowledge the contribution made by M. D. Kauffman, S. Roberts, M. Berg, and R. V. Vodraska on the writing of previous editions of this publication. The authors also wish to express their appreciation to the Soil Testing Laboratory personnel for their suggestions and contributions to this manual.

ANALYTICAL METHODS

pH
1:2 soil to water ratio

A. Reagents
Buffer solutions for calibration of pH meter.
Note: The buffer solutions can be purchased if desired.

1. pH 4.005 - 0.05 M potassium biphthalate ($KHC_8H_4O_4$). Dry $KHC_8H_4O_4$ for two hours at 110 C. Dissolve 10.21 g $KHC_8H_4O_4$ in distilled water and dilute the solution to a volume of 1 L with distilled water. As a preservative, add 1.0 mL chloroform or a crystal (about 10 mm in diameter) of thymol per liter of the buffer solution.
2. pH 6.860 - 0.025 M KH_2PO_4 and 0.025 M Na_2HPO_4. Dry the two phosphate salts for two hours at 110 C. Dissolve 3.40 g of KH_2PO_4 and 3.55 g of Na_2HPO_4 in distilled water and dilute the solution to a volume of 1 L with distilled water. As a preservative, add 1.0 mL of chloroform or a crystal (about 10 mm in diameter) thymol per liter of the buffer solution.
3. pH 9.177 - 0.01 M $Na_2B_4O_7$-$10H_2O$. Dry the $Na_2B_4O_7$-$10H_2O$ for two hours at 110C. Dissolve 3.81 g in distilled water and dilute the solution to 1 L.
4. Hydrochloric acid, 0.1 N HCl - Dilute 8.3 mL of concentrated HCl to 1 L volume with distilled water.

B. Procedure
1. Scoop 20 cc (g) of dry soil into a 3-oz paper cup or 100 mL beaker.
2. Add 40 mL of distilled water and stir thoroughly.
3. Let stand about 15 min, stir a second time, and allow suspended soil to settle for at least 15 min before reading pH.
4. Calibrate the pH meter according to instrument instructions using two of the prepared buffer solutions. After instrument calibration, rinse the electrodes with 0.1 N HCl and then distilled water to remove any trace of the buffer solutions.
5. Read the pH by placing the electrodes in the supernatant liquid and swirling gently. Record the pH to the nearest 0.1 unit.
6. Rinse the electrodes with distilled water and pat dry between pH determinations.
7. When the meter is not in use, immerse the electrodes in pH 6.860 buffer.
8. pH readings should be made routinely on known standard soil samples, every 15 samples in the OSUSTL.

C. Comments
This method is described by McLean (1982). The one used has a 1:2 soil-water ratio where the pH is measured in the supernatant instead of in the soil suspension, for convenience and to minimize the errors introduced by liquid junction potential.

Buffer solutions should be prepared fresh at least once a month. If solutions are purchased, expiration dates need to be noted. The pH meter needs to be calibrated periodically when making a series of determinations to check for drift. Check samples should also be incorporated into a series of analyses to ensure accurate readings. For pH measurements in soil a combination (single) or a dual electrode can be used. The OSUSTL uses a dual electrode.

Greweling and Peech (1968) indicate that pH may shift slightly with a change in the soil-to-water ratio used in sample preparation. Seasonal fluctuations in pH can also be expected. Soil pH will tend to be lower for samples collected after heavy fertilization. Conversely, pH may increase as the concentration of fertilizer salts decreases. Salt accumulation in soil tends to lower pH, and salt removal by leaching will have the opposite effect of raising pH. Fluctuations in pH due to seasonal or analytical effects may vary from 0.1 to 1.0 pH units.

Soil pH can also be determined using prepared salt solutions; this indicates the effect of salts in the sample. For example, the pH value obtained using 1 N KCl will normally be 1 to 1.5 units lower than the distilled water value. The soil pH measured in 0.01 M $CaCl_2$ will be about 0.4 to 0.8 units lower than in distilled water. Measuring soil pH in these salt solutions has the added advantage of maintaining flocculation which minimizes errors caused by liquid junction potentials

D. Equipment
1. pH meter with suitable electrode
2. Paper cups

LIME REQUIREMENT
SMP Buffer Method

A. Reagents
1. SMP buffer solution - Using a 1-L volumetric flask, completely dissolve 1.8 g of ground para-nitrophenol in 500 mL distilled water. Add 2.5 mL or 2.8 g of triethanolamine (weigh rather than pipette this viscous liquid). Then dissolve 3.0 g potassium chromate (K_2CrO_4), 2.0 g calcium acetate ($Ca(OAc)_2$-H_2O) and 53.1 g calcium chloride dihydrate ($CaCl_2$-$2H_2O$) in the solution. Bring to 975 mL volume with distilled water and stir overnight with magnetic stirrer. Adjust the solution to pH 7.5 with 0.1 N NaOH if necessary. Bring to 1 L volume with distilled water. This solution is usually made in 8 L quantities for convenience.

CAUTION: Trietanolamine and potassium chromate can be hazardous. Read label before use.

2. Sodium hydroxide, 0.1 N NaOH - Dissolve 4.0 g of NaOH pellets in about 500 mL distilled water. Allow to cool to room temperature and bring to 1 L volume.
3. Hydrochloric acid, 0.1 N HCl - Dilute 8.3 mL of concentrated HCl to 1 L volume with distilled water.
4. Phosphate buffer, pH 6.860 - See pH.

B. Procedure

1. Weigh 5.0 g of soil into paper cup or beaker. Generally samples are placed in rows of six to accommodate continuous stirring and reading of samples.
2. Add 5.0 mL of distilled water. Stir (leaving a stir rod in each sample) and allow to soak for 30 min.
3. Standardize the pH meter, described in B.4 of pH Procedure.
4. Add 10 mL of SMP buffer solution and stir every 5 min during the ensuing 20 minute period.
5. Immediately following the final stirring (20 min after addition of SMP buffer solution), insert the electrodes and observe the pH reading of the suspension, swirl gently and observe the subsequent reading. Continue until pH readings are constant, then record the pH reading to the nearest 0.1 unit.
6. Between readings, thoroughly rinse electrodes with distilled water and pat dry.

C. Comments

Reading the pH of the soil-buffer solution between 20 and 25 min after the addition of the SMP buffer is necessary because the pH of the suspension will continue to decrease over time. The electrodes should be rinsed with 0.1 N HCl and distilled water occasionally when making a series of determinations to eliminate increased pH readings caused by contamination of the electrodes.

The method outlined is a modification of the method described by McLean (1982).

D. Equipment

1. pH meter and suitable electrode
2. Paper cups

EXTRACTABLE PHOSPHORUS
Sodium Bicarbonate Method

Note: This method is used for all samples received from east of the Cascade Mountains.

A. Reagents

1. Sodium bicarbonate, 0.5 M $NaHCO_3$ - Using a 1-L volumetric flask, dissolve 42.01 g $NaHCO_3$ in 500 mL of distilled water and make up to volume. Cover and store overnight. Adjust the pH to 8.5 with 1 M NaOH. Cover the surface of the solution with an approximately 1 inch thick film of purified mineral (paraffin) oil to seal the solution from the air. When stored in a glass container, prepare a fresh solution monthly. A longer storage period is acceptable when the solution is stored in a polyethylene container. Check the pH of the solution each month, and adjust the pH if necessary. (See Section D, Comments.)
2. Ammonium paramolybdate - In a 1-L flask dissolve 15.0 g $(NH_4)_6Mo_7O_{24}$-$4H_2O$ in 300 mL of warm distilled water (60C). After cooling, filter the solution if turbidity is evident, adding 342 mL of concentrated HCl gradually while swirling; bring to volume. This solution contains enough concentrated HCl so that a 2 mL aliquot of ammonium paramolybdate solution has sufficient acid to neutralize the NaHCO in a 2 mL aliquot of soil extract.
3. Stannous chloride
   a. Stock solution - Dissolve 10.0 g $SnCl_2$-$2H_2O$ in 25 mL of concentrated HCl. Prepare fresh every two months or less. Use large reagent crystals for preparing the solution rather than fine powder, and store the stock solution in a refrigerator.
   b. Dilute solution - Add 0.5 mL aliquot of the stock solution to 66 mL of distilled water. Prepare this solution fresh daily.
4. Standard phosphate solutions
   a. Standard stock solution (50 ppm P) - Dissolve 0.2195g oven dried KH_2PO_4 in 500 mL distilled water and dilute to 1 L volume.
   b. Standard work solutions - Pipette the following aliquots of 50 ppm P stock solution into 100 mL volumetric flasks. Bring to volume with $NaHCO_3$ extracting solution.

mL stock solution	ppm P work solution
1	0.5
2	1.0
4	2.0
6	3.0
10	5.0

5. Sodium hydroxide, 1 M NaOH - Dissolve 40 g NaOH pellets in 500 mL distilled water and dilute to 1 L volume.

B. Procedure

1. Weigh or scoop 2.0 g of soil into a 50 mL extracting bottle and add 40 mL of $NaHCO_3$ extracting solution.
2. Shake the sample for 30 min, remove the sample from the shaker immediately after it stops. Decant the contents of the bottle into a filter funnel fitted with a Whatman No. 42 or equivalent filter paper. Refilter the extract if it is not clear.
3. Pipette 2.0 mL of the filtrate into a 25 mL colorimeter tube. Automatic pipettes are suitable for dispensing the small volumes used in all of the following steps of this procedure.
4. Add 2.0 mL of ammonium paramolybdate solution to each tube and mix well using a Vortex mixer. Remove all traces of the molybdate solution from the neck of the flask by washing with 5.0 mL of distilled water. Vortex for 5 s.
5. Add 0.5 mL of the dilute $SnCl_2$ solution, mix immediately.
6. Read color intensity in the colorimeter[3] set at a wavelength of 660 nm, at least 10 min but not more than 30 min after addition of the $SnCl_2$ solution.
7. Prepare a calibration curve using steps 3-6, but substitute 2.0 mL aliquots of the 0.5 to 5 ppm P standard solutions for the soil extract. Report the results in ppm P (mg kg^{-1}) in the soil sample.

C. Calculations

ppm P in the soil sample = ppm P in the soil extract x 20

D. Comments

This method for extractable P follows a procedure outlined by Olsen and Sommers (1982) with the following exceptions:

1. The ammonium paramolybdate solution contains sufficient HCl to neutralize the NaHCOa 2 mL aliquot of extractant. This eliminates the step of acidifying the aliquot with H_2SO_4.
2. A colorimeter tube is used for the color development step rather than a volumetric flask.
3. Stannous chloride is used as the reducing agent instead of ascorbic acid.

When P is extracted from soil with a 0.5 M $NaHCO_3$ solution at an approximate pH of 8.5, the concentrations of calcium (Ca), aluminum (Al), and iron (Fe) in solution are maintained at low levels. A decrease in activity or concentration of soluble Ca, Al, and Fe allows extraction of more soluble phosphate.

An increase in shaker speed or temperature of the extractant may cause an increase in P extracted from the sample. Normally, for routine testing, the extraction is performed at room temperature, though it may vary seasonally. The OSUSTL uses a constant-speed reciprocating shaker, which has a 2-inch stroke and operates at 200 oscillations per minute.

When exposed to the atmosphere, $NaHCO_3$-extracting solution increases over time. When pH of the extractant exceeds 8.5, an increase in extractable soil P is anticipated. Spreading a layer of mineral oil spread over the surface of the extracting solution will decrease the rate pH will change. Prolonged storage of the $NaHCO_3$ extractant in glass may also allow a pH increase. When glass storage vessels are used, check the pH of the solution at least monthly; if pH of the solution exceeds 8.5, prepare a new solution.

E. Equipment

1. Spectrophotometer
2. Flow-through cell or cuvettes
3. Extraction bottles
4. Filtration vials
5. Vortex mixer
6. Reciprocating shaker

EXTRACTABLE PHOSPHORUS
Dilute Acid-Fluoride Method (Bray-P1)

Note: This method is used for all samples received from west of the Cascade Mountains, including Hood River County.

A. Reagents

1. Ammonium fluoride, 1 N NH_4F - Dissolve 74 g of NH_4F in distilled water and dilute the solution to 2 L. Store the solution in a polyethylene bottle.
2. Hydrochloric acid, 0.5N HCl - Dilute 103 mL of concentrated HCl to a volume of 2500 mL with distilled water.
3. Extracting solution - Add 1350 mL of 1.0 N NH_4F and 2250 mL of 0.5 N HCl to 45 L of distilled water. This produces a solution of 0.03 N NH4F and 0.025 N HCL. It will keep indefinitely.
4. Standard phosphate solutions

   a. Standard stock solution, 100 ppm P - Dissolve 0.4393 g of oven dry KH_2PO_4 in 500 mL of distilled water and dilute to a volume of 1 L.

   b. Standard work solution - Pipette the following aliquots of 100 ppm stock solution into 100 mL volumetric flasks. Bring to volume with NH_4F extracting solution.

Aliquot mL	ppm P of solution
5	5
10	10
15	15
20	20

B. Procedure

(i) Automated Colorimetric Analysis (OSU Procedure)

1. Weigh 2.9 g (or scoop 2 g) of soil into a 50 mL extracting bottle and add 20 mL of the extracting solution.
2. Shake for 60 sec. and filter immediately using Whatman No. 42 or equivalent filter paper.
3. The concentration of P in the extract solution is determined on a ALPKEM rapid flow analyzer No. RFA-300 which relies on molybdate and antimony in acid to form a complex with ortho phosphate to yield a blue color.

(ii) Manual Colorimetric Analysis

1. Use same procedure as for sodium bicarbonate method.

C. Calculations

ppm P in soil sample = ppm P in soil extract x 7

D. Comments

The dilute acid-fluoride method for P follows a method described by Olsen and Sommer (1982). OSUSTL modifications are a 2.9 g weight used with a 60 second shaking time.

The dilute acid-fluoride extractant tends to dissolve Al and Fe phosphates in soil. The dissolution of Al and Fe phosphates occurs very rapidly and probably results from the fluoride anion complexing these metal cations in the acid solution. Interference in the development of the color complex occurs if appreciable amounts of Al, Fe (excess of 100 ppm), and molybdate are present. The fluoride ion may also interfere with color development when present in excess of 50 ppm. To minimize interferences, standards are made using the extracting solution.

E. Equipment

1. Auto-analyzer or spectrophotometer
2. Reciprocating shaker
3. Filtration vials
4. Extraction bottles

EXTRACTABLE CALCIUM, MAGNESIUM, POTASSIUM, AND SODIUM
Ammonium Acetate Method

A. Reagents

1. Ammonium acetate extracting solution, neutral, 1 N - Commercial ammonium acetate is purchased for ease of handling and to reduce ammonia contamination in the lab. To mix add 77.1 g ammonium acetate per liter of solution, usually mixed in 45 L quantities. This solution does not have to be neutralized as it does when acetic acid and ammonium hydroxide are used.
2. Lithium - lanthanum chloride solution (reagent grade $LaCl_3$-$7H_2O$ and LiCl), dissolve 200 g $LaCl_3$-$7H_2O$ and 50 g LiCl in a 22 L container with 5 L distilled water. Fill to the 22-L mark and mix.
3. Standard solutions

   a. Standard stock solutions. These can be prepared from commercial standard solutions which are available through most chemical suppliers, or can be prepared as follows:

   (i) Calcium (500 ppm Ca) - Dissolve 1.249 g of $CaCO_3$ in 1:1 HCl and evaporate to dryness on a hot plate. Dissolve the residue and bring to exactly 1 L with distilled water.

   (ii) Magnesium (500 ppm Mg) - Dissolve 0.50 g pure Mg ribbon in 1:1 HCl and evaporate to dryness on a hot plate. Dissolve the residue and then dilute to 1L with distilled water.

   (iii) Potassium (500 ppm K) - Prepare a standard solution of K by dissolving 0.9535 g oven dried KCl in a small volume of distilled water and diluting to 1 L with distilled water.

   (iv) Sodium (500 ppm Na) - Prepare a standard solution of Na by dissolving 1.271 g NaCl in a small volume of distilled water and diluting to 1 L with distilled water.

   b. Standard work solutions[4] K, Ca, Mg, and Na - Pipette the following aliquots of 500 ppm stock solutions into 100 mL volumetric flasks.

Dilutions of stock solutions for standard preparation.

Flask or	Ca		Mg	
Standard	Aliquot	ppm in	Aliquot	ppm in
No.	mL	solution	mL	solution
1	5	25	1.0	5.0
2	15	75	1.5	7.5
3	25	125	2.0	10.0
4	35	175	2.5	12.5
5	70	350	7.5	37.5

Flask or	Na		K	
Standard	Aliquot	ppm in	Aliquot	ppm in
No.	mL	solution	mL	solution
1	1	5	2	10
2	2	10	3	15
3	4	20	4	20
4	5	25	6	30
5	10	50	12	60

Bring to 100 mL volume with ammonium acetate. Mix thoroughly and store in plastic bottles.

B. Procedure

1. Weigh or scoop 2.0 g of soil into a 50-mL extracting vessel. Add 40 mL of the ammonium acetate extracting solution and place the extracting vessel containing the sample on the shaker for 30 min.
2. Filter through a Whatman No. 40 or equivalent filter paper.
3. K, Ca, Mg and Na. Using a Custom Lab Equipment diluter dispenser or the equivalent, dilute a 0.5 mL aliquot of the sample filtrate with 12 mL of $LaCl_3$-LiCl solution (a 25-fold dilution). Prepare standards by substituting 0.5 mL of standard K, Na, Ca or Mg work solutions for the sample filtrate. The blank is made by diluting the ammonium acetate extracting solution.
4. Calibrate the atomic absorption spectrophotometer[5] with the standard work solutions according to instrument instructions.
5. Report Ca, Mg, K and Na in millequivalents per 100 g, ppm or mg/kg of soil.

C. Calculations

ppm in the soil sample = ppm in the soil extract solution x 20

meq per 100g of sample = ppm in the soil sample divided by equivalent weight (K = 390, Ca = 200, Mg = 120, Na = 230)

D. Comments

The procedure for determining extractable cations with neutral 1 N ammonium acetate is a modification of the procedure outlined by Knudsen et al. (1982) for exchangeable K. The modification is the equilibration of a sample with one extracting solution (1:20 ratio of soil to extractant) rather than three different extractions, as specified in the original procedure. A further modification is the dilution of the soil extract with a joint lanthanum chloride and lithium chloride solution.

The single extraction technique for cations in non-calcareous soil results in values which are equivalent to at least 95% of the values obtained by the process of multiple extraction. For samples which contain carbonates of Ca or Mg, the multiple extraction with ammonium acetate may dissolve these carbonates and result in higher values for Ca and Mg than are obtained with a single extraction. However, for purposes of routine soil testing, there is usually no interest in determining the extractable Ca and Mg in alkaline samples which contain free lime.

Interferences caused by refractory compound formation and ionization are minimized by the dilution of the soil extract with lanthanum chloride and lithium chloride, respectively. The addition of lanthanum chloride minimizes the formation of Ca and Mg refractory compounds. Lithium chloride is added for Na and K determinations to minimize ionization interferences. In the past, these have been two separate solutions but it was determined that they could be mixed without sacrificing analytical accuracy. For some samples, the use of this mixture tends to stabilize readings and improve precision.

E. Equipment

1. Atomic absorption instrument
2. Filtration vials
3. Extraction bottles
4. Reciprocating shaker
5. Diluter-dispenser

mLs Stock II (5 ppm B)	Standard Work Solution (ppm B)
4	0.20
8	0.40
12	0.60
20	1.00
28	1.40
40	2.00

HOT-WATER EXTRACTABLE BORON
Azomethine H Method

A. Reagents

1. Buffer masking agent - Completely dissolve 250 g ammonium acetate (reagent grade $NH_4C_2H_3O_2$), 25 g tetrasodium salt of ethylene-dinitrillotetraacetic acid (Na_4-EDTA), and 10 g disodium salt of nitrilotriacetic acid (Na_2-NTA) in 400 mL distilled water in a 1-L beaker using a magnetic stirrer. Add 125 mL glacial acetic acid very slowly, while stirring. The temporary acidic conditions may cause a slight precipitation of the EDTA salts. Continue to stir the solution until all the EDTA redissolves. Do not heat the solution. Adjust the buffer to a pH of 5.4 to 5.6 with acetic acid or ammonium hydroxide as necessary. If the spectrophotometer is equipped with an aspirating flow-cell, add six drops of Brij-35 surfactant (ALPKEM) to 250 mL buffer masking agent. Prepare this solution every two months.
2. Azomethine-H solution - Dissolve 0.9 g azomethine-H reagent (Pierce Chemical Co., Rockford, IL) and 2.0 g ascorbic acid ($C_6H_8O_6$) in about 50 mL of distilled water. A hot tap water bath facilitates dissolution. Bring to 100 mL volume with distilled water. Prepare this solution fresh daily.
   Note: Azomethine-H reagent may also be prepared in the laboratory.
3. Calcium chloride extracting solution, 0.02 M - Dissolve 2.84 g calcium chloride dihydrate ($CaCl_2$-$2H_2O$) in about 700 mL distilled water, then bring to one liter volume. Store in plastic container.
4. Boron standard solutions - All standard solutions should be stored in plastic bottles.
   a. Standard solution I, 500 ppm B - Pipette 5.0 mL of 5000 ppm aqueous boron standard solution (available commercially) into a 50 mL volumetric flask. Bring to volume with distilled water. A 500 ppm B standard solution can also be prepared by dissolving 0.8820 g oven-dry re-crystallized sodium tetraborate (reagent $Na_2B_4O_7$-$10H_2O$) in distilled water and diluting to 200 mL.
   b. Standard solution II, 5 ppm B - Pipette 5.0 mL of standard solution I (500 ppm B) into a 500 mL volumetric flask. Bring to volume with distilled water.
   c. Standard work solution - Prepare work solutions by pipetting the following aliquots of standard solution II (5 ppm B) into 100 mL volumetric flasks. Bring to volume with $CaCl_2$ extracting solution.

B. Procedure

1. Weigh or scoop 15 g of soil into a sealable plastic bag (heat sealed boilable bags or ziplock freezer bags work). Add 30 mL of $CaCl_2$ extracting solution.
2. Place plastic bags into boiling water and leave for 10 min. The OSUSTL uses a porcelain canning pot with cover.
3. Remove plastic bags, let cool to room temperature and filter the contents through a Whatman No. 42 or equivalent filter paper.
4. Pipette 4.0 mL of soil extract into a 12 mL polyethylene sample vial.
5. Add 1 mL of buffer masking agent and vortex.
6. Add 1 mL of azomethine-H solution and vortex. Allow color to develop for at least 1 hour but no longer than 3 hours.
7. Prepare standard curve following steps 4-6, substituting 4.0 mL of standard work solution for soil extract. A blank is prepared in the same manner using 4.0 mL $CaCl_2$ extracting solution instead of the soil extract.
8. For samples with a yellow extract: Prepare a second sample solution and blank following steps 4 and 5. Add 1.0 mL of distilled water in place of azomethine-H solution and vortex well. The blank for this determination consists of 5.0 mL $CaCl_2$ extracting solution and 1.0 mL buffer masking agent.
9. Read all color intensities on a spectrophotometer set at 420 nm. Read immediately after vortexing.

C. Calculations

ppm B in soil = (ppm B extract - ppm B in yellow extract) x2

D. Comments

A method described by Bingham (1982) is used here with adaptation to the use of plastic bags as described by Mahler et al. (1983). It was determined that plastic bags are more suitable and less expensive than boron free glassware, which is no longer obtainable. The pH of the buffer was originally prescribed as 5.2, but 5.4 to 5.6 is adequate. Further reductions in pH only increases the difficulty of keeping the EDTA in solution.

The EDTA and NTA chelates eliminate interferences from Al, Fe, and Cu. The concentration of these chelates should be effective for levels of these elements commonly found in soil extracts.

The azomethine-H should be added quickly so that time for color development is equal for all tubes. A constant check must be maintained on linearity and drift of the standard curve when analyzing a large batch of samples. Correction for a yellow extract as described here is probably legitimate for only

a mild yellow color and is insufficient for some of the deep brown or yellow extracts occasionally obtained. For these ICP analysis is preferable. Acid washing of all glassware is recommended to minimize the potentials for boron contamination.

D. Equipment

1. Spectrophotometer
2. Flow through cell or cuvettes
3. Filtration vials
4. Hot plate and boiling container with cover
5. Vortex stirrer

ORGANIC MATTER
Walkley-Black Method

A. Reagents

1. Potassium dichromate, 1 N $K_2Cr_2O_7$ - Dissolve 49.04 g of reagent grade $K_2Cr_2O_7$ in 500 mL distilled water and dilute the solution to a volume of 1 L.
2. Ferrous ammonium sulfate, 0.4 N $Fe(NH_4)_2(SO_4)_2$-$6H_2O$ - Dilute 40 mL concentrated H_2SO_4 in 500 mL distilled water. Dissolve 159.6 g $Fe(NH_4)_2(SO_4)_2$6-H_2O in the acid solution; cool the solution and dilute it to a volume of 1 L. Determine the normality periodically by titrating against the $K_2Cr_2O_7$ solution. Store in opaque bottle as light affects this solution.
3. O-phenanthroline ferrous sulfate complex indicator, 0.025 M-This solution is also referred to as 1,10 phenanthroline iron (II) sulfate and is commercially available under the trade name "Ferroin."
4. Phosphoric acid, 85 percent, H_3PO_4.
5. Sulfuric acid, concentrated, not less than 96 percent H_2SO_4.

B. Procedure

1. Pass the soil sample through a 0.5 mm sieve and weigh out 0.50 g of soil into a 500-mL Erlenmeyer flask.
2. Add 10 mL of 1 N $K_2Cr_2O_7$ solution and swirl the flask to gently disperse the soil in the solution. Take care not to throw sample onto sides of flask.
3. Rapidly add 20 mL of concentrated H_2SO_4. Swirl for 10 seconds. Let cool uniformly to room temperature, at least 20 min. 4. Dilute to approximately 150 mL with distilled water and add 10 mL of concentrated H_3PO_4. The addition of H_3PO_4 is optional and the OSUSTL omits this step for routine analysis.
5. Add 6 drops of O-phenanthroline indicator to the solution. Titrate with the ferrous ammonium sulfate solution (FAS) until the color changes from yellow or yellow-green to blue to finally a reddish brown endpoint. Record the volume (mLs) of FAS used to reach the endpoint.
6. Analyze a blank simultaneously following steps 2-5.

C. Calculation

Calculate the percent organic matter as follows:

$$\%OM = (\text{Blank-reading}) \times \frac{13.6}{\text{blank}}$$

Calculate the percent organic carbon as follows:

$$\%OC = \%OM \times 0.58$$

D. Comments

The wet oxidation method for determining organic matter in soil is the same method as described by Nelson and Sommers (1982). The only modification involves the use of the O-phenanthroline in place of the diphenylamine indicator.

Grinding of the soil sample to pass a 0.5 mm sieve facilitates obtaining a representative subsample, increasing surface area and ridding the sample of ground plant material. If more than 75% to 80% of the total dichromate reagent is reduced by the oxidizable material in the sample, the entire analysis must be repeated using a smaller soil sample.

The soil is digested with the dichromate and sulfuric acid mixture by the heat of dilution. For precise results the sulfuric acid should be added rapidly and the flasks should be cooled uniformly. Once these steps are accomplished, variations in reaction time from 20 to 40 min do not appreciably affect the results.

For soils or other materials high in organic matter, the organic matter content may be more accurately determined using the Ignition method presented in the Appendix.

E. Equipment

1. Titration apparatus
2. Lighted stirring plate

SOLUBLE SALTS
Electrical Conductivity Method

A. Reagent

1. Potassium chloride reference solution, 0.01 N KCl - Dissolve 0.7456 g of KCl in distilled water and dilute the solution to a volume of 1 L at 25 C. This solution has a conductivity of 1.4118 mmhos per cm (ds/m).

B. Procedure

1. Place 30 to 50 mL of soil in a 10 oz paper cup; add distilled water while stirring to prepare a saturated soil paste. (At saturation, the soil paste glistens as it reflects light and it flows slightly when the container is tipped. The paste slides freely and cleanly off the spatula unless the soil has a high clay content.)
2. Allow the saturated soil to stand at least 30 min. Then ascertain that the above criteria for saturation are still evident. Free water should not collect on the soil surface, nor should the paste stiffen markedly or lose its glisten. Remix the sample, if necessary, by adding either additional water or soil to obtain a saturated paste.
3. Transfer the saturated soil paste to a Buchner funnel fitted with a Whatman No. 42 filter. By vacuum filtration[6], collect an aliquot of the saturation extract in a 25 mL receiving flask.
4. Using the reference solution, calibrate the conductivity meter[7] according to instrument instructions.
5. Record the electrical conductivity (EC) reading for the saturation extract when it has reached the same temperature as the reference solution.

C. Comments

The procedure for determining total soluble salts follows closely a method described by Rhoades (1982b). For an appraisal of soil salinity, the extraction can usually be made a few minutes after the saturated paste is prepared. The recommended time lapse between preparation of the soil paste and extraction is several hours for gypsiferous samples and from 4 to 16 hr in all cases where the chemical constituents are to be determined in the extract. Determination of chemical constituents in the extract requires a larger soil sample (200-400 g soil) than for soluble salts alone. If the initial filtrate is turbid, it can be discarded or refiltered through a clean sheet of filter paper.

The Solu-Bridge used in the OSUSTL is designed specifically for determining the conductivity of saturation extracts. When the compensator dial is set on the temperature of the solution, the conductivity dial at balance indicates directly the electrical conductivity at 25 C. A calculation to obtain the result is unnecessary.

E. Equipment

1. Conductivity meter
2. Suction filtration apparatus

CATION EXCHANGE CAPACITY (CEC)
Ammonium Acetate Method

A. Reagents

1. Ammonium acetate extracting solution, neutral, 1 N - Prepare according to the specifications outlined in the ammonium acetate method for extractable cations.
2. Ethanol, 95%
3. Hydrochloric acid, 0.1 N HCl - Dilute 8.3 mL of concentrated HCl reagent to 1 L with distilled water.

B. Procedure

1. Weigh 10 g of soil into a 125 mL Erlenmeyer flask; add 50 mL of ammonium acetate solution and place the flask containing the sample on the shaker for 30 min.
2. Connect a 1-L vacuum extraction flask to a Buchner funnel fitted with a Whatman No. 5 or equivalent filter paper. Moisten the filter paper with distilled water.
3. Transfer the soil suspension into the Buchner funnel and leach the sample with 175 mL of 1 N ammonium acetate. This soil extract may be analyzed for extractable K, Ca, Mg, and Na.
4. Rinse the excess ammonium acetate from the soil sample in the Buchner funnel by leaching with a total volume of ethanol and discard the leachate. Note: Be sure to gently fill funnel to remove all excess ammonium and allow it to drain until only damp soil remains. Continue adding alcohol in this manner until 200 mL of ethanol has been used.
5. Change to a clean 500-mL suction flask and leach the soil sample with 225 mL of 0.1 N HCl to replace the exchangeable ammonium. Bring leachate to volume in a 250 mL volumetric flask using distilled water.
6. The concentration of ammonium-N in the final leachate is determined with an ALPKEM rapid flow analyzer (RF-300), which relies on ammonium to complex with salicylate to form indophenol blue (Technicon Method No. 334-74A/A). This color is intensified with sodium nitroprusside and measured at 660 nm. This determination can also be made using the Kjeldahl distillation method (see Appendix).

C. Calculation

$$\text{CEC in meq per 100 g of soil} = (\text{ppm } NH_4\text{-N in leachate}) \times \frac{0.25}{14} \times \frac{100}{\text{sample size (g)}}$$

ppm NH_4-N in leachate is determined using a standard curve.

D. Comments

The procedure used is essentially the same as that of Schollenberger (1945) except that determination of NH_4-N is done spectrophotometrically rather than by Kjeldahl distillation and titration. To determine the NH_4-N content using the Kjeldahl distillation method, follow steps 1-5 above, then proceed to Appendix. Care must be taken not to allow soil to dry and

crack between alcohol leachings, as this could result in incomplete removal of excess NH_4-N. A similar procedure is described by Rhoades (1982a).

E. Equipment

1. Buchner funnels and source of vacuum
2. Auto analyzer or Kjeldahl distillation equipment
3. Vacuum flasks

TOTAL NITROGEN (TN)
Kjeldahl Method

A. Reagents

1. Sulfuric acid, concentrated H_2SO_4 - reagent grade
2. Digestion catalyst - Mix together 1000 g of ground sodium sulfate (reagent anhydrous Na_2SO_4) or potassium sulfate, 25 g cupric sulfate (reagent anhydrous $CuSO_4$), and 10 g of reagent selenium (Se) powder. Packets of prepared catalyst can be purchased.

CAUTION: DO NOT BREATHE $CuSO_4$ and Se dust..

B. Procedure

1. Weigh 3.0 g of soil into a 75 mL volumetric digestion tube. Use 1.0 g of soil if sample is greater than approximately 20% in organic matter.
2. Add a 3 g scoop of digestion catalyst and mix thoroughly with the dry soil.
3. Add 10 mL of concentrated H_2SO_4 to the soil-catalyst mixture. ***Note: It is essential that all dry material be completely moistened and well mixed with the acid to insure complete digestion.***
4. Prepare a blank with each set of samples analyzed by following steps 2-3 above using no soil. Allow the samples and blank to stand overnight.
5. Place tubes on a digestion block[8] at 150 C. Check samples every 20 min for foaming. After one hour (or more if foaming persists), raise temperature to 250 C, and continue digestion for one hour. After one hour at 250 C raise temperature to 350 C and heat until samples are completely digested, usually about two additional hours. At completion, mineral soils will be greyish-white while organic soils will be blue-green in color.
6. Remove samples from block and leave under a fume hood until cool. Then add 10-20 mL distilled water to each tube to keep samples from hardening.
7. The ammonium-N content of the digest solution is determined with an ALPKEM rapid flow analyzer (RF-300) which relies on ammonium to complex with salicylate to form indophenol blue (Technicon Method No.334-74A/A). This color is intensified with sodium nitroprusside and measured at 660 nm. This determination can also be made using the Kjeldahl distillation method (see Appendix). For samples to be analyzed on an auto analyzer, continue with steps 8-9 and determine total N using calculation in Part C.
8. Bring samples to volume with deionized water in 75 mL digestion tubes and mix.
9. Obtain a clear digest solution for analysis either by allowing samples to settle overnight and pipetting an aliquot or by filtering through an acid washed filtering apparatus fitted with Whatman No. 042 or equivalent filter paper. Digest solutions may be refrigerated prior to analysis.

C. Calculation

$$\% \text{ Total Nitrogen} = (\text{ppm } NH_4^+\text{-N in digest solution}) \times \frac{75 \text{ mL}}{\text{sample size (g)}} \times \frac{1}{10{,}000}$$

D. Comments

The Kjeldahl method outlined by Bremner and Mulvaney (1982) is modified by eliminating the water from the digestion step. One further modification is the determination of NH_4-N spectrophotometrically rather than by Kjeldahl distillation and titration. To determine the NH_4-N concentration using the Kjeldahl distillation method, follow steps 1-6 and then proceed to Appendix.

E. Equipment

1. Digestion block
2. Digestion tubes
3. Autoanalyzer or Kjeldahl distillation unit

AMMONIUM AND NITRATE NITROGEN
KCl Extraction Method

A. Reagents

1. Potassium chloride extracting solution, approximately 2 N KCl - Dissolve 150 g of reagent KCl in 500 mL distilled water and dilute to a volume of 1 L.

B. Procedure

1. Place 20 g of soil into a 250 mL extracting bottle and add 75 mL of 2 N KCl extracting solution. ***Note: If using the Kjeldahl distillation method, add 150 mL of extracting solution.*** Shake the vessel on a mechanical shaker for one hour. Remove from shaker and allow the soil-KCl suspension to settle (about 30 min).
2. Filter the extract solution through Whatman No. 42 or equivalent filter paper. To minimize contamination by filter paper, it is first leached with 20-50 mL of KCl solution. If the extract cannot be analyzed on the same day as prepared, store in a refrigerator or freezer until analysis can be performed.
3. The ammonium-N content of the extract is determined with an ALPKEM rapid flow analyzer (RF-300) which relies on ammonium to complex with salicylate to form indophenol blue (Technicon Method No. 334-74A/A). This color is intensified with sodium nitroprusside measured at 660 nm. This determination can also be made using the Kjeldahl distillation method (see Appendix).

4. The nitrate-N content of the extract is determined with an ALPKEM rapid flow analyzer (RF-300) which reduces nitrate to nitrite via a cadmium reactor then complexes nitrite with sulfanilamide and N-(1-Napthyl)-ethylenediamine dihydrochloride to form a red-purple color that is measured at 540 nm (Technicon Method No. 329-74W/A). This determination can also be made using the Kjeldahl distillation method (see Appendix).

C. Calculation

ppm NH_4-N or NO_3-N in soil sample =
(ppm NH_4-N or NO_3-N in filtrate x 3.75)

D. Comments

The method outlined by Keeney and Nelson (1982) for determining ammonium and nitrate-N is used with a modification in which 75 mL of KCl and 20 g of soil are used instead of 100 mL and 10 g soil. To determine NH_4-N or NO_3-N concentration using the Kjeldahl method, follow steps 1-2 and then proceed to Appendix.

The extended period of shaking the soil sample with 2 N KCl according to the specifications of Bremner's original procedure permits the simultaneous extraction of ammonium and nitrate.

E. Equipment

1. Autoanalyzer or Kjeldahl distillation apparatus
2. Reciprocating shaker
3. Filtration Vials
4. Extraction Bottles

EXTRACTABLE ZINC, COPPER, AND MANGANESE
DTPA Method

A. Reagents

1. Diethylenetriaminepentaacetic acid, 0.025 M DTPA - Mix 9.83 g DTPA in glass-distilled water and dilute to a volume of 1 L.
2. Triethanolamine, 0.5 M TEA - Mix 74.60 g TEA in glass-distilled water and dilute to a volume of 1 L.
3. Calcium chloride, 0.05 M $CaCl_2$ - Dissolve 5.55 g anhydrous $CaCl_2$ in glass-distilled water and dilute to 1 L.
4. DTPA extracting solution, 0.05 M DTPA, 0.1 M TEA, and 0.01 M $CaCl_2$ - Combine reagents from steps 1, 2, and 3, and dilute to 5 L with glass-distilled water. Adjust the resulting solution after it has set for 12 hr to pH 7.3 with concentrated HCl. Two mL of concentrated HCl is needed to change the pH of the DTPA solution 0.1 units. Store the solution in the refrigerator.
5. **Standard solutions**

a. Standard stock solutions - These are easily made from commercial standard solutions which are available through most chemical suppliers, or can be prepared as follows:
(i) Zinc (100 ppm Zn) - Weigh 0.1000 g of pure Zn metal (30-mesh, analytical reagent) into a 1-L volumetric flask. Add 50 mL of Zn-free water and 1 mL of concentrated H_2SO_4. When the Zn has dissolved, make to volume with DTPA extracting solution.
(ii) Copper (100 ppm Cu) - Dissolve exactly 0.1000 g of pure metallic Cu in 15 mL of 3 N HNO_3 at room temperature in a covered 125-mL Erlenmeyer flask. When the solution has cooled, add 1 mL of concentrated H_2SO_4 and evaporate the solution cautiously until SO_4 fumes are evolved. Cool the solution again; dilute it cautiously with 10 to 15 mL of glass distilled water and again evaporate until it fumes SO_4. Finally, when the solution has cooled, dilute it cautiously with water, transfer it quantitatively to a 1-L flask and dilute the solution to volume with DTPA extracting solution.
(iii) Manganese (100 ppm Mn) - Dissolve 0.2880 g of dry, pure $KMnO_4$ in about 250 mL of H_2O in a 1-L beaker. Add 20 mL of 18 N H_2SO_4; heat the solution to boiling. Add solid Na_2SO_4 until the color of permanganate disappears (avoid a large excess of Na_2SO_4) and boil off the SO_2. Cool the solution, transfer to a 1-L volumetric flask, and bring to volume with DTPA extracting solution.

b. Standard work solutions - Prepare standard work solutions by pipetting the following amounts of 100 ppm standard stock solutions into 100 mL volumetric flasks and diluting to volume with DTPA extracting solution:

Dilutions of stock solutions for metal standard preparation.

Zn		Cu		Mn	
mL 100 ppm Zn	ppm Zn in solution	mL 100 ppm Cu	ppm Cu in solution	mL 100 ppm Mn	ppm Mn in solution
0.5	0.50	1.0	1.00	1.0	1.00
1.0	1.00	2.0	2.00	3.0	3.00
3.0	3.00	5.0	5.00	9.0	9.00

B. Procedure

1. Weigh 10 g of soil into a 125 mL Erlenmeyer flask.
2. Add 20 mL of DTPA extracting solution.
3. Shake on mechanical shaker for two hours at a speed fast enough to keep soil in suspension.
4. Immediately filter through a Whatman No. 42 or equivalent filter paper. Refilter if filtrate is cloudy.
5. Calibrate the atomic absorption spectrophotometer in accordance with instrument instructions using the prepared standard work solutions. The blank is DTPA extracting solution.
6. Determine the concentration of Zn, Cu, and Mn in the filtrate and report as ppm metal in the soil on a dry weight basis.

C. Calculations

ppm Zn in soil sample = ppm Zn in soil extract x 2

D. Comments

The following precautions are essential to avoid problems of contamination in conducting analyses: (1) All solutions should be prepared with glass-distilled water; (2) All glassware is rinsed with .5 N HCl and then rinsed with glass-distilled water; (3) The filter paper should be checked continuously for presence of zinc, copper, and manganese by analyzing a blank that has been filtered.

The DTPA soil test was developed to measure the availability of zinc, copper, manganese, and iron for plant uptake (Lindsay and Norvell, 1978). Since there have been few reported iron deficiencies in Oregon, the OSU soil testing lab does not routinely measure this nutrient in the extract.

E. Equipment

1. Filtration vials
2. Extraction bottles
3. Reciprocating shaker
4. Atomic absorption spectrophotometer

SULFATE SULFUR (SO_4-S)
Ion Chromatograph Method

A. Reagents

1. Standard sulfate-S solutions

   a. Standard stock solution, 100 ppm SO_4-S - Dissolve 0.5434 g of oven dry potassium sulfate (K_2SO_4) in 500 mL distilled water and dilute to a volume of 1 L.

   b. Standard working solutions - Prepare work solutions by pipetting the following aliquots of 100 ppm SO_4-S stock solution into 100 mL volumetric flasks. Bring to volume with calcium phosphate extracting solution. The standards are adjusted to suspected concentration of the samples being analyzed. For example, if a sample has a concentration of 3 ppm (.3 ppm in extract) then a standard curve may be developed at .1, .3, .7, and 2 ppm SO_4.

mL 100 ppm stock solution	ppm SO_4-S in work solution
1	1
3	3
7	7
10	10
20	20

2. Calcium phosphate extracting solution, 500 ppm PO_4 - Dissolve 2.17 g calcium phosphate ($Ca(H_2PO_4)_2$) in 500 mL distilled water and dilute to 1 L volume.

B. Procedure

1. Extraction of SO_4-S
   a. Weigh 5 g of soil into a 100 mL glass or plastic bottle.
   b. Add 50 mL of extracting solution and shake vigorously enough to keep soil suspended for 1 hr.
   c. Filter through Whatman No. 42 filter paper (or equivalent).
2. Determination of SO_4-S Inject 50 uL of extract into ion chromatograph (dionex 2000i) equipped with AS4A anion exchange column with flow rate set at 2 mL per min. The sulfate peak elutes between 6 and 8 minutes.

C. Calculations

Peak height is integrated by computer and compared to known standards to yield concentration of SO_4 in the extraction solution.

Soil concentration in ppm SO_4 is then calculated by multiplying solution concentration by ten.

D. Comments

The use of an ion chromatograph for sulfate analysis has been shown to be comparable to the methylene blue method (Dick and Tabatabai, 1979). The use of an ion chromatograph also yields greater precision and accuracy than other procedures, especially at low concentrations. The methylene blue method, recommended if access to an ion chromatograph is not available, is described in the Appendix.

EXCHANGEABLE SODIUM
Ammonium Acetate Displacement Method[9]

A. Reagents

1. Ammonium acetate extracting solution, neutral, 1 N - Use the same solution prepared for determining ammonium acetate extractable cations.
2. Standard solution, 500 ppm sodium (Na) - Use the same solution which was prepared for determining ammonium acetate extractable Na in the extractable cations section.

B. Procedure

1. Weigh 5 g of soil into a 50-mL plastic centrifuge tube.
2. Add 10 mL of distilled water.
3. Shake by hand three or four times during a 5 to 10-min period to mix.
4. Centrifuge to clarify. Decant supernatant liquid into a paper cup. Test conductivity of supernatant liquid. If over 1.1 mmhos/cm, add 10 mL of distilled water and repeat dilutions until conductivity is below 1.1.
5. Using a stainless steel spatula to loosen the soil in the tube, quantitatively transfer the soil into a 125-mL Erlenmeyer flask using exactly 100 mL of ammonium acetate extracting solution.
6. Swirl every five minutes during a half-hour period.
7. Filter through a Whatman No. 40 or equivalent filter paper.
8. Determine the concentration of Na in the soil extract by the same atomic absorption procedure used to determine ammonium acetate extractable Na.
9. Report the results as exchangeable Na in milliequivalents (meq) per 100 g of soil.

C. Calculations

meq of exchangeable Na per 100 g of soil sample =
ppm of Na in extract x 0.087 (x additional dilution if necessary)

D. Comments

All soil samples should be washed at least once with distilled water to remove any soluble Na. After most of the soluble Na is removed by washing, the conductivity of the wash water should be reduced to approximately 0.9 to 1.1 mmhos/cm (ds/m). The ammonium acetate extractable Na is determined and regarded as an estimate of exchangeable Na. An estimate of exchangeable Na in conjunction with the value for cation exchange capacity serves as a basis for predicting the quantity of soil amendments needed to reclaim sodic soils.

EXCHANGEABLE HYDROGEN
Barium Chloride-Triethanolamine Method

A. Reagents

1. Buffer solution, approximately 0.5 N barium chloride ($BaCl_2$-$2H_2O$) and 0.2 N triethanolamine (TEA) - Prepare the following solutions (a and b) and mix together. Protect the buffer solution from CO_2 contamination by storing in a tightly closed plastic container or attaching a tube containing soda lime to the air intake.
   a. TEA, 0.4 N - Mix 50 mL (56.3 g) of TEA (specific gravity 1.125, about 8N) in 500 mL of distilled water. Partially neutralize the pH to 8.1-8.3 using approximately 150 mL of 1.0 N HCl. Dilute this solution to a volume of 1 L with distilled water.
   b. BaCl2, 1.0 N - Dissolve 125 g $BaCl_2$-$2H_2O$ in 500 mL distilled water and then dilute to a volume of 1 L.
2. Replacement solution, 0.5 N $BaCl_2 2H_2O$ in dilute buffer solution - Dissolve 250 g of $BaCl_2$-$2H_2O$ in 2 L of distilled water and dilute to a 4 L volume. Then mix with 20 mL of buffer solution (Reagent 1).
3. Hydrochloric acid, 0.3 N HCl, standardized - Dilute 24.9 mL of reagent concentrated HCl to 1 L with distilled water. Standardize against 0.1000 N sodium carbonate (Na_2CO_3) or 0.1000 N sodium hydroxide (NaOH). These standard base solutions are available through most chemical suppliers, or can be prepared from pure, dry reagent Na_2CO_3 or NaOH. See Appendix for general standardization procedure.
4. Mixed indicator - Dissolve 0.1 g of bromocresol green and 0.02 g of methyl red indicators in 75 mL of 95% ethyl alcohol, then bring to 100 mL volume.

B. Procedure

1. Place at least 10 g of soil in a 125-mL Erlenmeyer flask. *Note: With soils having very high acidity, use 5 g and adjust calculation accordingly.*
2. Add 25 mL of buffer solution and swirl the flask occasionally during a 30-minute period to mix the sample suspension.
3. Fit a Buchner funnel which contains a Whatman No. 42 or equivalent paper to a 500-mL vacuum extraction flask. Moisten filter paper with a small amount of buffer solution.
4. Transfer the sample suspension to the Buchner funnel using an additional 25 mL of buffer solution to completely remove sample from the original 125-mL Erlenmeyer flask. Adjust the filtration rate so that this filtration step requires at least 30 min.
5. When the buffer solution has leached through and only damp soil remains, leach the soil sample with an additional 100 mL of the replacement solution (Reagent 2) by repeatedly adding small increments of the solution to the sample in the funnel.
6. When leaching is completed, remove suction flask and add 10 drops of mixed indicator to the filtrate. Titrate with standardized 0.3 N HCl to a faint pink endpoint. Record the mLs of acid used to reach the endpoint.
7. Titrate a blank solution which contains 50 mL of buffer solution to the same endpoint selected for the sample. The blank determination serves as a reference for the calculation.

C. Calculation

Calculate the result as follows from the volume of standardized HCl used:

Exchangeable hydrogen in meq per 100 g of soil sample =

$$\frac{(\text{mL HCl}_{\text{blank}} - \text{mL HCl}_{\text{sample}}) \times \text{N HCl} \times 100}{10\ (\text{g of sample})}$$

D. Comments

The $BaCl_2$-TEA method for determination of exchangeable H as described by Thomas (1982) is followed except for the following modifications:

1. 0.3 N HCl is used instead of 0.2 N HCl.
2. After addition of 25 mL buffer solution into 10 g of soil, the flask is occasionally swirled over a 30 minute period rather than allowing the mixture to stand for 1 hour.
3. Only 25 mL of additional buffer solution is added to remove sample from the original 125-mL Erlenmeyer flask instead of 75 mL of buffer solution.
4. The mixed indicator is slightly different.

This procedure is used as a research tool and is not performed on a routine basis in the OSUSTL.

At the endpoint of the titration, the mixed indicator changes from blue-green through violet and finally to pink. Any stage of the progressive color change may be selected as the endpoint; but the blank and the samples must be titrated to the same endpoint.

The $BaCl_2$-TEA extraction estimates the total "potential" acidity which may be related to a potential liming level and a potential CEC. Thomas suggested the use of a KCl extraction method which estimates the neutral and salt-exchangeable acidity. The KCl method is thought to be related to the immediate need for lime and an existing CEC.

E. Equipment

1. Extraction flasks
2. Buchner funnels
3. Vacuum source
4. Titration equipment

CARBONATE
Titrimetric Method

A. Reagents

1. Hydrochloric acid, 2 N HCl - Add 167 mL of concentrated HCl to about 700 mL of distilled water and then dilute to a volume of 1 L.
2. HCl, 1 N - Add 83 mL of concentrated HCl to about 700 mL of distilled water and then dilute to a volume of 1 L.
3. HCl, 0.1 N standardized - Dilute 8.3 mL of concentrated HCl to a volume of 1 L with distilled water. See Appendix for general standardizing instructions.
4. Potassium hydroxide, 2 N KOH - Dissolve 132 g of KOH (85%) in about 700 mL of distilled water and dilute to a volume of 1 L. Protect the solution from atmospheric CO_2 by storing in a tightly stoppered bottle.
5. Bromocresol green indicator - Dissolve 0.1 g of bromocresol green in 100 mL of 95% ethanol.
6. Phenolphthalein indicator - Dissolve 0.05 g of phenolphthalein in 50 mL of ethanol. Add 50 mL of distilled water and mix well.

B. Procedure

1. Weigh 3.0 g of soil into a 250-mL Erlenmeyer flask (or 8 oz French square bottle). If the needle-puncture stopper pops off the glass tube following the addition of the 2 N HCl (Step 4), use 2.0 g of soil. The amount of soil can be further reduced if needed, to as little as 0.5 g. If the stopper pops when using 0.5 g of soil, use the $CaCO_3$ equivalent procedure used for liming materials, in Appendix.
2. Connect a 5.0 mL beaker to the glass tube below the stopper about 5 mm above the lower end of the tube. Pipette 4.0 mL of 2 M KOH into the 5.0 mL beaker.
3. After stoppering the flask, remove 50 mL of air from the flask via the needle-puncture stopper using a 50-mL gas syringe. Be sure the stopper has been resealed.
4. Inject 20 mL of 2 N HCl into the flask via the needle-puncture stopper with a 20 mL syringe. Be sure stopper has resealed. Swirl the flask gently to mix contents, being careful not to spill the KOH.
5. Allow the flask to stand at room temperature (20-25 C) for 16 to 24 hrs. Then quantitatively transfer the contents of the 5.0 mL beaker into a 125-mL Erlenmeyer flask using 50 mL of distilled water.
6. Add 6 drops of phenolphthalein indicator to the flask and titrate with 1 N HCl until the pink color begins to fade. At this point, titrate with 0.1 N HCl until the solution turns colorless. It is advisable to do one sample at a time, as the pink color of the phenolphthalein tends to fade with time.
7. Add 8 drops of bromocresol green indicator and titrate with the standardized 0.1 N HCl to a pale-yellow endpoint.
8. Determine a blank by following the procedures in the above analysis except do not add soil.

C. Calculations

Inorganic carbonate expressed as percent $CaCO_3$ =

$$\frac{[(\text{mL HCl}_{\text{sample}} - \text{mL HCl}_{\text{blank}}) \times N \times 0.100]}{\text{wt. of soil sample}} \times 100$$

where mL HCl refers to the amount of acid titrated following the addition of the mixed bromocresol green indicator.

D. Comments

This method follows the same procedure as presented by Bundy and Bremner (1972), except 4 mL of KOH is used instead of 3 mL KOH; N-octyl alcohol is not used and the bromocresol green indicator is made up with ethanol rather than NaOH. These changes should not significantly affect the results.

This procedure determines total carbonate which may be present in compounds such as calcium carbonate, magnesium carbonate and various bicarbonates.

MINERALIZABLE NITROGEN
Anaerobic Incubation

A. Reagents

1. Potassium chloride, 2 N KCl - Dissolve 150.0 g of KCl in about 500 mL distilled water and dilute to a volume of 1L.

B. Procedure

1. Using a sample splitter, obtain a soil sample of at least 20 g. Weigh 20.0 g of sample into a 125-mL extraction bottle.
2. Add 25.0 mL of distilled water and stir well with a glass rod to insure that the soil is completely wet. Add another 25.0 mL of distilled water to rinse glass rod and side of jar.
3. Place a sheet of parafilm, then a layer of plastic wrap over the mouth of the bottle and tightly secure the lid. Place in an incubator set at 40 plus or minus 0.5 C for 7 days (168 hr).
4. Remove samples from incubator and carefully add 50.0 mL of 2 N KCl. Replace the plastic covers and tighten lid securely.
5. Shake briskly to disperse the soil and place on a mechanical shaker for 1 hour. Filter through a Whatman No. 42 or equivalent filter paper into acid-rinsed filter vials.
6. Determine the NH_4-N content of the extract solution from the incubated sample on an automated colorimetric analyzer. This determination can also be made using the Kjeldahl distillation-titration method, described in Appendix.
7. Determine the initial NH_4-N (reference) content in the soil by following steps 1-2 and 4-6 above.

C. Calculations

ppm mineralizable NH_4-N = (ppm NH_4-N in incubated extract - ppm NH_4-N in reference extract) x 5

D. Comments

This procedure is a modification of the anaerobic incubation described by Keeney (1982). Sample size has been increased from 5 to 20 g. A 125-mL screw-top extracting bottle is used here to accommodate the larger sample size and volume of solutions.

Because of the biological nature of this procedure, there is a higher level of variability in the results than in many other soil testing procedures. Therefore, all attempts to reduce variation are critical. To reduce experimental error, the following are recommended: thorough sample mixing, complete sealing of bottles during incubation, avoidance of floating particles during incubation, and strict temperature control. Preliminary results showed no advantage in excluding oxygen from the headspace by introducing a N_2 atmosphere immediately prior to sealing of the incubation vessel. Keeney and Bremner (1966) reported the erratic results whenever the smell of H_2S was detected during analysis.

The mineralizable NH_4-N content of some soils has been found to vary with time in dry storage. The OSUSTL currently recommends holding samples in dry storage for a minimum of three weeks before analysis. It is also recommended that samples be rapidly air-dried at ambient temperature immediately after sampling.

WATER ANALYSIS METHODS

Irrigation Water Quality

CALCIUM, MAGNESIUM, AND SODIUM

A. Reagents

Same as used for Extractable Bases.

B. Procedure

1 Filter through Whatman No. 42 or equivalent filter paper.
2. Dilute and analyze sample filtrate following steps 3-5 of the Extractable Bases procedure.

C. Calculations

$$\text{meq of cation/liter} = \frac{\text{ppm (mg/L) of cation in sample}}{\text{meq weight of cation}}$$

BORON

A. Reagents

Same as used for soil boron test.

B. Procedure

1. Add 2 drops of $CaCl_2$ extracting solution to about 30 mL of the water sample. Allow to stand for 5-10 min.
2. Filter through Whatman No. 42 or equivalent filter paper.
3. Follow steps 4-9 of the Hot-Water Soluble Boron procedure for soils, substituting the water sample for the soil extract.

C. Calculations

ppm B in water sample = ppm B in water - ppm B in yellow colored sample (if any)

SALINITY

A. Reagent

1. Potassium chloride solution 0.01 N. See Soluble Salts for soils.

B. Procedure

1. Calibrate the solu-bridge with .01 N KCl by placing instrument indicator on 1.41 and turning the temperature indicator until red and green lights are of equal intensity (same as step B.4, in Soluable Salts).
2. Record the electrical conductivity reading for each sample.

pH

A. Reagents

Same as used for soil pH test.

B. Procedure

Same as used for soil pH test except use 40 mL of water sample and omit steps 1-3.

CARBONATES AND BICARBONATES

A. Reagent

1. Hydrochloric acid, 0.1 N standardized HCl - Dilute 8.3 mL of concentrated HCl to a volume of 1 L using distilled water.
2. Phenolphthalein indicator: Dissolve 0.05 g of phenolphthalein in 50 mL of 95% ethanol and dilute to a volume of 100 mL using distilled water. Mix well.
3. Mixed indicator: Dissolve 0.1 g bromocresol green and 0.02 g of methyl red indicators in 100 mL of 95% ethanol.

B. Procedure

1. Pipette 50 mL of water sample into a 125 mL Erlenmeyer flask.
2. Add 6 drops of phenolphthalein indicator.
3. Titrate with 0.1 N standardized HCl until the indicator changes from a pink color to a clear end point. If solution remains clear after addition of phenolphthalein then proceed directly to the second titration (step 4).
4. Add 6 drops mixed indicator and titrate with 0.1 N standardized HCl to a pale pink end point.

C. Calculations

1. First titration (step 3)
   meq carbonate/liter = mL of HCl x 2 x N of HCl x 20
2. Second titration (step 4)
   meq carbonate + bicarbonate/liter = mL of HCl x N of HCl x 20

SULFATE SULFUR

A. Reagents

Reagents will be the same as for the soil SO4-S test except that calcium phosphate solution is not required.

B. Procedure

Follow steps of the soil SO_4-S test.

C. Calculations

Determine the amount of SO_4-S from a standard curve prepared from a series of standard solutions.

TOTAL NITROGEN
Kjeldahl Procedure

A. Reagents

Same used for soil TN.

B. Procedure

1. Pipette a 10.0 mL aliquot of the water sample into a 75 mL volumetric digestion flask.
2. Follow steps 2-8 of the soil Total Nitrogen procedure. The samples will be a clear blue-green color when digested. A blank should be run using 10 mL of distilled water.

C. Calculation

$$\text{ppm total nitrogen} = \text{ppm NH4-N in filtrate} \times \frac{75}{\text{sample size (mL)}}$$

AMMONIUM AND NITRATE NITROGEN
KCl Extraction Method

A. Reagents

None.

B. Procedures

1. Follow steps 2-3 of the Extractable Ammonium and Nitrate Nitrogen procedure substituting an aliquot of water sample for the KCl extract solution. The Kjeldahl distillation method requires a 50-mL aliquot of water.
2. If determinations are to be made by Kjeldahl distillation, follow the procedural steps outlined for ammonium and nitrate nitrogen in steps 3a-i.

C. Calculation

For samples analyzed with an automatic analyzer, ppm ammonium-N or nitrate-N in solution is determined directly.

NOTES

1. Distributed by Custom Laboratory Equipment, Inc., Orange City, FL.

2. The Bausch and Lomb "Spectronic 88 spectrophotometer is used in OSUSTL.

3. Some changes in the concentrations of the standard work solutions may be required to insure operation within the linear range of the spectrophotometer being used.

4. A Perkin-Elmer model 372 atomic absorption spectrophotometer is used in the OSUSTL.

5. The five-unit vacuum filtering rack used in the OSUSTL is supplied by Soil Test, Inc., Evanston, IL.

6. RD-26 Solu-Bridge, Industrial Instruments, Cedar Grove, NJ, is used in the OSUSTL.

7. A Technicon 40-position digestion unit is used in the OSUSTL (Technicon, Inc.).

8. From an unpublished procedure entitled, "A Gypsum Requirement Test, Determination of Sodium in Equilibrium Ammonium Acetate Solution," supplied by Dr. A. R. Halvorson, Extension Soils Specialist, Washington State University, Pullman.

9. In this laboratory, heating mantels and rheostat set at 90.

10. From an unpublished procedure entitled, "Procedure for Purifying Activated Charcoal," which was supplied by Dr. A. R. Halvorson, Extension Soils Specialist, Washington State University, Pullman, WA.

11. Distributed by Custom Laboratory Equipment, Inc., Orange City, FL.

12. Some changes in the concentrations of the standard work solutions may be required to insure operation within the linear range of the spectrophotometer.

13. The five-unit vacuum filtering rack used in the OSUSTL is supplied by Soil Test, Inc., Evanston, IL.

14. RD-26 Solu Bridge, Industrial Instruments, Cedar Grove, NJ, is used in the OSUSTL.

15. From an unpublished procedure entitled, "A Gypsum Requirement Test, Determination of Sodium in Equilibrium Ammonium Acetate Solution," supplied by Dr. A. R. Halvorson, Extension Soils Specialist, Washington State University, Pullman, WA.

16. All glassware should be acid washed and rinsed with glass-distilled water.

17. OSUSTL heating mantels and rheostats are set at 90.

18. From an unpublished procedure entitled, "Procedure for Purifying Activated Charcoal," which was supplied by Dr. A. R. Halvorson, Extension Soils Specialist, Washington State University, Pullman, WA.

APPENDIX*

ORGANIC MATTER
Ignition Method

A. Reagents

None

B. Procedure

1. Tare a 50-mL beaker or crucible by igniting it in a muffle furnace set at 550 C, cooling it in a desiccator, and weighing it to plus or minus 1 mg (tare).
2. Place 10-20 g of air-dried soil into the tared container and place in a drying oven set at 100 C for 2-3 hr. Cool container in a desiccator and weigh (soil).
3. Place the container plus sample in a muffle furnace set at 550 C for 4-5 hr. Cool container in a desiccator and weigh (burn).

C. Calculation

$$\% \text{ O.M.} = \frac{\text{soil - burn}}{\text{soil - tare}} \times 100$$

D. Comments

This method appears to be superior to the Walkley-Black method for samples high in organic matter. However, hydrated aluminosilicates, loose structural water, and carbonate minerals are decomposed upon heating which may result in weight losses in excess of the actual organic matter content. The method outlined by Nelson and Sommers (1982) in Section 29-4.3 suggests pretreatment of the soil with a mixture of HCl and HF to remove the hydrated mineral matter. Samples containing carbonate minerals should be pretreated with HCl to dissolve all of the carbonates. To test for the presence of carbonates follow the procedure below:

> Place small amount of finely ground soil on a sheet of wax paper and moisten with a few drops of water. Add approximately 4 N HCl drop-wise to the moist sample, and note any evidence of effervescence. Allow sufficient time to react.

KJELDAHL DISTILLATION
CEC, TN, NH_4-N, NO_3-N and Mineralizable-N

A. Reagents

1. Mixed Indicator - Dissolve 0.3 g of bromocresol green and 0.165 g of methyl red indicators in 400 mL of 95% ethanol, and bring to 500 mL volume.
2. Boric acid indicator, 4% H_3BO_3 - Dissolve 20 g of reagent grade H_3BO_3 in about 900 mL distilled water; heat and swirl until dissolved. Add 20 mL of mixed indicator (reagent 1). Adjust to reddish-purple color or until 1 mL water added to 1 mL solution turns indicator a light green. Adjust indicator solution with 0.1 N sodium hydroxide (NaOH) (pH around 5.0) and dilute to 1 L.
3. Sodium hydroxide, 40% NaOH - Dissolve 400 g of NaOH pellets in about 500 mL distilled water. Cool and bring to 1 L volume.
4. Sodium chloride (NaCl) - Reagent grade, granular.
5. Devarda's alloy - Grind reagent grade alloy in a ball mill until it will pass a 100-mesh sieve and 75% will pass a 200-mesh sieve.
6. Magnesium oxide - Oven dry heavy magnesium oxide (MgO) in a muffle furnace at 650 C for 2 hr. Cool and store in a desiccator.
7. Hydrochloric acid, 0.1 N, standardized - Add 8.3 mL of concentrated HCl to 500 mL distilled water, then bring to 1 L volume. Standardize following the general procedure outlined in Appendix. This is used for titrations in the determination of cation exchange capacity and total nitrogen.
8. Hydrochloric acid, 0.01 N, standardized - Dilute 100 mL of 0.1 N HCl with distilled water to a volume of 1 L. Standardize following the procedure outlined in Appendix. This is used for titrations in the determination of ammonium and nitrate nitrogen.

B. Procedure

1. Turn on heating unit to boiling flask and condensers.
2. Pipette 10 mL of boric acid indicator solution into a 125 mL Erlenmeyer flask. Place the Erlenmeyer flask under the condenser tip of the Kjeldahl unit. The end of the condenser should be in the boric acid indicator. Make sure the system is boiling before attaching the Kjeldahl flask to the distillation system in Step 3.

(Note: Steps 1 and 2 precede all succeeding steps.)

* The appendix contains a combination of alternate procedures, seldom used procedures and instructions for standardization of an acid.

CEC

3. Transfer a 50 mL aliquot of leachate from CEC step 5 into a 300-mL Kjeldahl flask. Add 3 g of NaCl to leachate in flask. Place flask on system.
4. Add 20 mL of 40% NaOH to the leachate through the stopcock; rinse with a small amount of distilled water, and close the stopcock.

Note: It is advisable to turn the steam off before adding reagents through the stopcock to avoid spitting. Be sure to turn the system back on before plugging the stopcock.

5. Distill approximately 75 mL into the 125-mL Erlenmeyer flask containing the boric acid indicator. Remove the steam bypass plug and then remove the Erlenmeyer flask.
6. Titrate with 0.100 N HCl to a pink endpoint.
7. Make a blank determination following the same procedure as the samples using 50 mL of 0.1 N HCl in place of the leachate.

TN

3. Quantitatively transfer the contents of the 75-mL volumetric digestion tube into a 300-mL Kjeldahl flask and attach to distillation system.
4. Add 30 mL of 40% NaOH to the digested solution through the stop cock, rinse with a small amount of distilled water and close the stop cock. *(See Note in CEC.)*
5. Follow Step 5 in CEC distillation.
6. Titrate with 0.1 N HCl to a pink endpoint.
7. Make a blank determination on sample that was digested with each set of samples following the same procedure only without adding soil.

Extractable NH_4-N and NO_3-N

3. Transfer a 50-mL aliquot of the filtered KCl extract solution into a 300-mL Kjeldahl flask.

NH_4-N Determination

4. Add 0.8 g MgO directly to the Kjeldahl flask and immediately attach to the distillation unit.
5. Follow Step 5 in CEC distillation.
6. Titrate with 0.01 N HCl to a pink endpoint.
7. Make a blank determination following the same procedure, using 50 mL of 1 N KCl in place of the sample filtrate.

NO_3-N Determination (Nitrite is also analyzed)

4. After removal of NH_4-N from the sample as described in the previous section, replace the Erlenmeyer flask with one containing fresh boric acid indicator (Step 2). Then add 0.8 g of Devarda alloy through the stopcock; rinse with a small amount of distilled water and close the stopcock.
5. Follow Step 5 in CEC distillation.
6. Make a blank determination following the same procedure, using 50 mL of 1 N KCl in place of the sample filtrate.

NO_3-N and NH_4-N Determination

4. Follow the same procedure described for determination of NH_4-N, but add 0.8 g of Devarda alloy to the distillation chamber immediately after addition of MgO.

Washing of Kjeldahl distillation unit.

a. Fill a Kjeldahl flask with 1 N HCl. Attach to the Kjeldahl distillation unit, insert the steam bypass stopcock, and turn on the steam generator unit.
b. Allow the acid to boil over through the condenser until thoroughly flushed. Remove the plug, then remove the Kjeldahl flask.
c. Repeat steps a and b above using distilled water.

Note: Washing is necessary to remove any traces of Devarda's alloy which may accumulate. The presence of the alloy will cause a negative error in the NO_3-N determination.

D. Calculations

1. Cation Exchange Capacity in meq/100 g soil =

$$\frac{(\text{mL HCl sample} - \text{mL HCl blank}) \times \text{N of HCl} \times 5 \times 100}{\text{soil sample size (g)}}$$

2. % Total Nitrogen in soil =

$$\frac{(\text{mL HCl sample} - \text{mL blank}) \times \text{N of HCl} \times 0.014\,\text{g N/meq}}{\text{soil sample size (g)}}$$

3. ppm NH_4-N or NO_3-N is soil =

$$\frac{(\text{mL HCl sample} - \text{mL blank}) \times \text{N of HCl} \times 0.014\,\text{g N/meq}}{\text{soil sample size (g)} \times \left(\frac{\text{mL of aliquot}}{\text{mL of extract}}\right)}$$

E. Comments

Some of the reagents used in the Kjeldahl distillation determinations have been modified from the method presented by Bremner and Mulvaney (1982). These modifications have been developed so that the procedure can be used for routine soil analysis.

SULFATE SULFUR (SO_4-S) Distillation Method

A. Reagents[10]

1. Reducing agent - Under a fume hood, mix 400 mL of hydriodic acid (56%), 100 mL hypophosphorus acid (50%), and 200 mL formic acid (88%) in a sturdy 1000 mL beaker. Boil gently with a stream of nitrogen flowing through this solution for about 10 min after the temperature has reached 115 C. The nitrogen gas should be bubbled through the solution by passing N_2 through a glass tube placed near the bottom of the beaker. Do not let the temperature of the solution exceed 117 C. Do not attempt to recover spent reagent by distillation. Remove beaker from hot plate and maintain N_2 flow through the solution until cool. Store in glass container. Reagent is stable for two months.

CAUTION! EXTREMELY POISONOUS FUMES OF PHOSPHINE (PH_3) may be liberated from the reagent if heated above 120 C or if the reagent is spilled on a hot surface.

2. **Pyrogallol - sodium phosphate wash solution** (Not used unless solution contains high levels of NO_3)
   a. Stock reagents
      (i) Dissolve 100 g of sodium phosphate monobasic (NaH_2PO_4-H_2O) in 500 mL glass-distilled water and dilute to 1 L volume.
      (ii) Crush about 100 g of crystalline pyrogallol [pyrogallic acid, $C_6H_3(OH)_3$] using a mortar and pestle. Store in a tightly closed container.
   b. Working wash solution
      (i) Weigh 1+ g of crushed pyrogallol into a 150 mL beaker for each distillation unit to be used (e.g., 6 g for a 5-unit system).
      (ii) Saturate the atmosphere in the beaker with N_2 gas. This can be accomplished by holding the end of a tygon tube from which an audible stream of N_2 gas is flowing near the bottom of the beaker for about 1 minute.
      (iii) Add 12 mL of sodium phosphate monobasic solution per distillation unit to the beaker and stir with a magnetic stirrer until the pyrogallol is dissolved. An atmosphere of N_2 gas needs to be maintained above the solution to prevent the pyrogallol from being oxidized and turning yellow.
3. **Zinc acetate-sodium acetate**(sulfide absorbing solution) - Dissolve 50 g of zinc acetate dihydrate ($Zn(CH_3COO)_2$-$2H_2O$) and 12.5 g of sodium acetate trihydrate (CH_3COONa-$3H_2O$) in 500 mL glass-distilled water then dilute to 1 L volume. A bulk supply of a dilute zinc acetate-sodium acetate can be made by diluting the above solution to a 7 L volume with glass-distilled water.
4. **Amino dimethylaniline solution** - Dissolve 2.0 g of p-amino dimethylaniline sulfate in 1500 mL of glass-distilled water. Slowly add 400 mL of concentrated reagent grade sulfuric acid (H_2SO_4) inside cold, running water bath to cool and avoid evaporation. Dilute the cooled solution to 2 L with glass-distilled water.
5. **Ferric ammonium sulfate solution** - Add 15 mL of concentrated H_2SO_4 to 75 g of ferric ammonium sulfate crystals [$FeNH_4(SO_4)_2$- $12H_2O$]. Add 585 mL of glass-distilled water slowly without mixing to keep H_2SO_4 on bottom and to allow dissolution of ferric ammonium sulfate. The crystals dissolve in around 10 days.
6. **Standard sulfate-S solutions**
   a. Standard stock solution, 100 ppm SO_4-S - Dissolve 0.5434 g of oven dry potassium sulfate (K_2SO_4) in 500 mL glass-distilled water and dilute to a volume of 1 L.
   b. Standard working solutions - Prepare work solutions by pipetting the following aliquots of 100 ppm SO_4-S stock solution into 100 mL volumetric flasks (bring to volume with the appropriate potassium chloride extracting solution):

mL 100 ppm stock solution	ppm SO_4-S in work solution
1	1
3	3
7	7
10	10
15	15

7. **Potassium chloride extracting solutions** -
   a. Eastern Oregon: 1 N KCl - Dissolve 74.56 g potassium chloride (KCl) in 500 mL of glass-distilled water and dilute to 1 L volume.
   b. Western Oregon: 1 N KCl + KH_2PO_4 - Dissolve 4.39 g KH_2PO_4 and 74.56 g KCl and bring up to 2 L with glass-distilled water.
8. **Nitrogen gas** (prepure)
9. **Sulfur-free ground joint lubricant** - Most ground joint lubricants contain appreciable sulfur that must be removed before use. Many lubricants deteriorate quickly when exposed to the hot reducing agent. Dow-Corning silicone stopcock lubricant has been found suitable if freed from sulfur contaminant. Place about 5 g of the silicone lubricant in a 100-mL beaker, add 5 mL of hydriodic acid and 5 mL of hypophosphorous acid. Place a watch glass filled with distilled water on top of the beaker to act as a condenser. Boil the mixture gently with frequent stirrings for about 45 min. Allow to cool, pour off the acid mixture, and wash the lubricant thoroughly with glass-distilled water.

B. Procedure

1. Extraction of SO^4-S
   a. Weigh 10 g of soil into a 50 mL plastic bottle.
   b. Add 20 mL of the appropriate KCl extracting solution and shake for one hour. The shaking action should be sufficiently vigorous to keep the soil suspended in solution.
   c. Filter through Whatman No. 42 filter paper (or equivalent).
2. Preparation of digestion-distillation apparatus
   a. Rinse washing columns with 0.5 N NaOH and then glass-distilled water.
   b. Lubricate all spherical joints with a minimal amount of S-free lubricant.
   c. Saturate the column with N_2 gas to reduce the possibility of oxidizing the pyrogallol. Place 10 mL of the pyrogallol-sodium phosphate wash solution in the gas washing column, then resaturate the column and solution with N_2 gas. Plain water may be used in gas traps unless solutions contain high levels of nitrate. Reattach the columns to the apparatus.
   d. Saturate the system (digestion-distillation apparatus and washing solution) with H_2S by using a 15 ppm SO_4-S standard solution. Follow sulphur determinate described below with the following exception: Vent H_2S-N_2 into the hood when the system is being saturated.

Note: Saturation should be done prior to analyzing samples each day or when new solution is introduced. The solution should be changed when yellow color appears or when the system has been used 25-30 times.

3. **Determination of SO_4-S**

a. Place 50 mL of the dilute zinc acetate-sodium acetate solution into a 100-mL volumetric receiving flask. Connect the glass delivery tube to the side arm of the gas washing column. Place the receiving flask with the delivery tube inside and near the bottom of the receiving flask, but not touching it.

b. Pipette a 2.0 mL aliquot of standard solution or sample extract into a 50 mL digestion-distillation flask and add 4 mL of reducing reagent. It is recommended that this and all succeeding steps (3b through 3h) be conducted under a suitable fume hood.

c. After moistening joint with a drop of water to insure a complete seal, immediately attach the digestion-distillation flask to the condenser and connect the nitrogen supply tube. Adjust the N_2 flow rate to about 2 bubbles per second. Make certain cool water is passing through the condenser.

d. After 5 min of N_2 flow to obtain a reduced atmosphere, apply heat to the digestion-distillation flasks by either lighting suitable microburners or positioning preheated heating mantels around the base of the flask. With N_2 still flowing, heat the contents of the flask and maintain at a low boil[11] for one hour.

e. Remove the receiving flask, leaving the glass delivery tube in the zinc acetate solution. Immediately add 10 mL of the amino dimethylaniline solution. Quickly stopper the receiving flask and mix thoroughly.

f. Add 2 mL of ferric ammonium sulfate solution and shake. Allow blue color to develop for at least 1/2 hr but no longer than 10 hr. Dilute to a 100 mL volume with glass-distilled water and mix thoroughly, leaving glass tube inside.

g. The blue color developed will be quite stable after 30 min. and should be read within 24 hr on a suitable spectrophotometer set at 670 nm.

h. Prepare standards following steps 3a-g, substituting 2.0 mL of the standard work solutions for the soil extract. A blank is prepared in the same manner using 2.0 mL of the appropriate extracting solution instead of soil extract.

i. If the color is more intense than that obtained for the highest standard work solution, make an appropriate dilution. For best results, dilute the soil extract to a concentration within the linear range of standard work solutions using the appropriate KCl extracting solution and following steps 3a-g.

C. Calculations

ppm SO_4-S in soil sample = ppm SO_4-S in soil extract x 2

D. Comments

The methylene blue method for the determination of sulfur as described by Tabatabai (1982) is followed except for the following modifications:

1. A special technique is used to make up the pyrogallol-sodium phosphate wash solution. When the wash solution is prepared in the manner described above, up to 25 determinations can be made before the solution becomes discolored.
2. The zinc acetate-sodium acetate is made up in the dilute form.

The methylene blue method used here yields more accurate values than the turbidimetric procedure of Tabatabai and Bremner (1970). A modified turbidimetric method has also been used for sulfur analysis but is not described here.

CALCIUM CARBONATE EQUIVALENT FOR LIMING MATERIALS AND HIGHLY BASIC SOILS

A. Reagents

1. Hydrochloric acid, 0.500 N HCl, standardized - Dilute 46.5 mL concentrated HCl to a volume of 1 L with distilled water. Standardize against 25 mL of 0.500 N sodium carbonate (Na_2CO_3) or sodium hydroxide (NaOH). These standard base solutions are available through most chemical suppliers, or can be prepared from pure, dry reagent Na_2CO_3 or NaOH.
2. Sodium hydroxide, 0.500 N NaOH, standardized - Dissolve 20.00 g NaOH pellets in about 500 mL distilled water. Cool and dilute to a volume of 1 L. Standardize against the 0.500 N standard HCl (reagent 1).
3. Phenolphthalein indicator - Dissolve 0.05 g phenolphthalein in 50 mL of 95% ethanol. Bring to 100 mL volume with distilled water.

B. Procedure

1. Place 1.0 g of ground liming material or 5 to 10 g of soil in a 150-mL Erlenmeyer flask. To initially determine how much soil to use, add a drop of 0.5 N HCl to some of the soil. If the soil effervesces, 5 g should be used.
2. Add 50.0 mL of the standardized 0.5 N HCl to the Erlenmeyer flask and boil gently for 5 min. A watch glass filled with cool distilled water placed on top of the flask will act as a condenser.
3. Allow the solution to cool. Rinse any condensation on the watch glass into the solution with distilled water. For soil, filter through a Whatman No. 42 or equivalent filter paper into a 250-mL flask, washing all soil from the Erlenmeyer flask with distilled water.
4. Titrate the excess acid with the standardized 0.5 N NaOH, using 4 drops of phenolphthalein indicator. The end point will be pink.

C. Calculations

$$\% \text{ calcium carbonate equivalent} = \frac{(\text{mL of HCL x N of HCl}) - (\text{mL of NaOH x N of Na OH}) \times 0.05}{\text{sample size (g)}}$$

D. Comments

The above test should be used for materials with percent calcium carbonate greater than 20. If percent calcium carbonate is less than 20, use the carbonate method found on p. 12. The above method does not differentiate between calcium and magnesium carbonates.

STANDARDIZATION OF ACID

A. Reagents

1. Sodium carbonate, 0.1 N (Na_2CO_3)
2. Acid - Acid of unknown normality to be standardized.
3. Mixed indicator - Dissolve 0.1 g of bromocresol green and 0.02 g of methyl red indicators in 75 mL of 95% ethyl alcohol, then bring to 100 mL volume.

B. Procedure

1. Pipette a known amount of 0.1 N Na_2CO_3 into a 100-mL beaker.
   Note: Use 10 mL for acid around 1.0 N, and 1.0 mL for acid around 0.1N.
2. Add 5 drops of mixed indicator.
3. Titrate with the unknown acid to a pink endpoint.
4. Calculate the normality of the acid.

C. Calculation

$$\text{Normality of acid} = \frac{(\text{N of } Na_2CO_3)\ (\text{mL } Na_2CO_3)}{\text{mL of acid used to titrate}}$$

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Appendix 3B

Quality Control At OSU Soil Testing Laboratory

DEPARTMENT OF CROP AND SOIL SCIENCE

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Quality Control at OSU Soil Testing Laboratory

February 20, 1991
By Donald A. Horneck

It is the policy of the OSU Soil Testing Laboratory (OSU-STL) to take the necessary steps to insure that quality results are maintained. This is done in many ways in the laboratory. First of all the OSU-STL participates in a northwest regional sampling program operated through Utah State University. This program allows us to compare our results with other laboratories throughout the region. For data regarding results from this program please contact the Soil Testing office.

General

To insure unbiased analysis and accurate record keeping, samples are assigned a unique lab number, which is written with a permanent marker on each sample bag. Batches of samples are brought up on trays of 32. The first and 16th soil samples on each tray are internal lab standards. The 32nd sample on each tray is duplicated. Samples on each tray are given a consecutive number to minimize the potential of samples getting out of order. Glassware is arranged and numbered in racks of eleven, three racks to a tray.

Soil reference samples are collected, ground and mixed. They are analyzed with a previously established reference sample to determine values. The results from the reference samples are recorded every time they are analyzed and kept on file. Tolerances are set, generally one standard deviation. Reference samples are evaluated when a batch of samples is run. The reference sample is used as a way of insuring that samples are in their correct order and that procedures are operating correctly.

More information as to how instruments are calibrated, samples are prepared and solutions are mixed can be found in our methods manual.

Bases - K, Ca, Mg, Na

Several steps are taken to insure accurate results. The instrument (Perkin Elmer 372) is calibrated every time it is used and when elements are changed. The five point plus a blank standard curve is recorded so that day to day fluctuations are known. When running a batch a point on the curve is checked every 11 samples with the whole curve checked after every tray (33 samples).

Samples are diluted with a lanthanum and lithium solution which minimizes interferences and gives a uniform salt background. Lanthanum is added to eliminate interferences with calcium and magnesium. Lithium is added to prevent ionization of sodium and potassium.

Standards are mixed from purchased solutions that are traceable to NBS standards and diluted in the same way that samples are handled. Reference samples are run every 15 samples and a duplicate every 30 samples.

Phosphorous (Bray)

Phosphorous is run on a continuous flow analyzer (Alpkem RFA) using the molybdate blue method with an in line-dialyzer. A standard curve consisting of four points and a blank is run every 35 samples. A constant check is maintained on baseline drift. Multiple sampling is done where increased precision is needed. Reference samples are analyzed every 15 samples and a duplicate every 30 samples.

Constant shaking times are maintained. Colloidal contamination is visually evaluated after filtration and samples are refiltered when necessary.

Organic Matter or Carbon (OM, OC)

Samples are hand ground to pass a 0.50 mm sieve to insure that fresh organic material is excluded and help increase surface area for reaction. Normality of the titrant is checked (blank) every 20 samples. Reference soil samples are analyzed with every blank and recorded.

pH

Samples are scooped and read in exact tray order. The pH meter is calibrated with purchased buffer solutions that are traceable to NBS standards. Reference samples are run every 15 samples and a duplicate every 30 samples.

Nitrates and/or Ammonium

Samples are weighed into numbered bottles. Filter paper is leached first with 50-100 ml KCl prior to filtration of sample to minimize contamination from filter paper. Reference samples and blanks are analyzed a minimum of every 25 samples.

Nitrate and ammonium are run on a continuous flow analyzer (Alpkem RFA) using cadmium reduction and indophenol methods, respectively. An in line-dialyzer is also used. A standard curve consisting of four points and a blank points is run every 35 samples. A constant check is maintained on baseline drift. Multiple sampling is done where increased precision is needed. Reference samples are analyzed every 15 samples and a duplicate every 30 samples.

Constant shaking times are maintained. Colloidal contamination is visually evaluated after filtration and samples are refiltered when necessary.

Figure 1 table 2. Project QA Organization

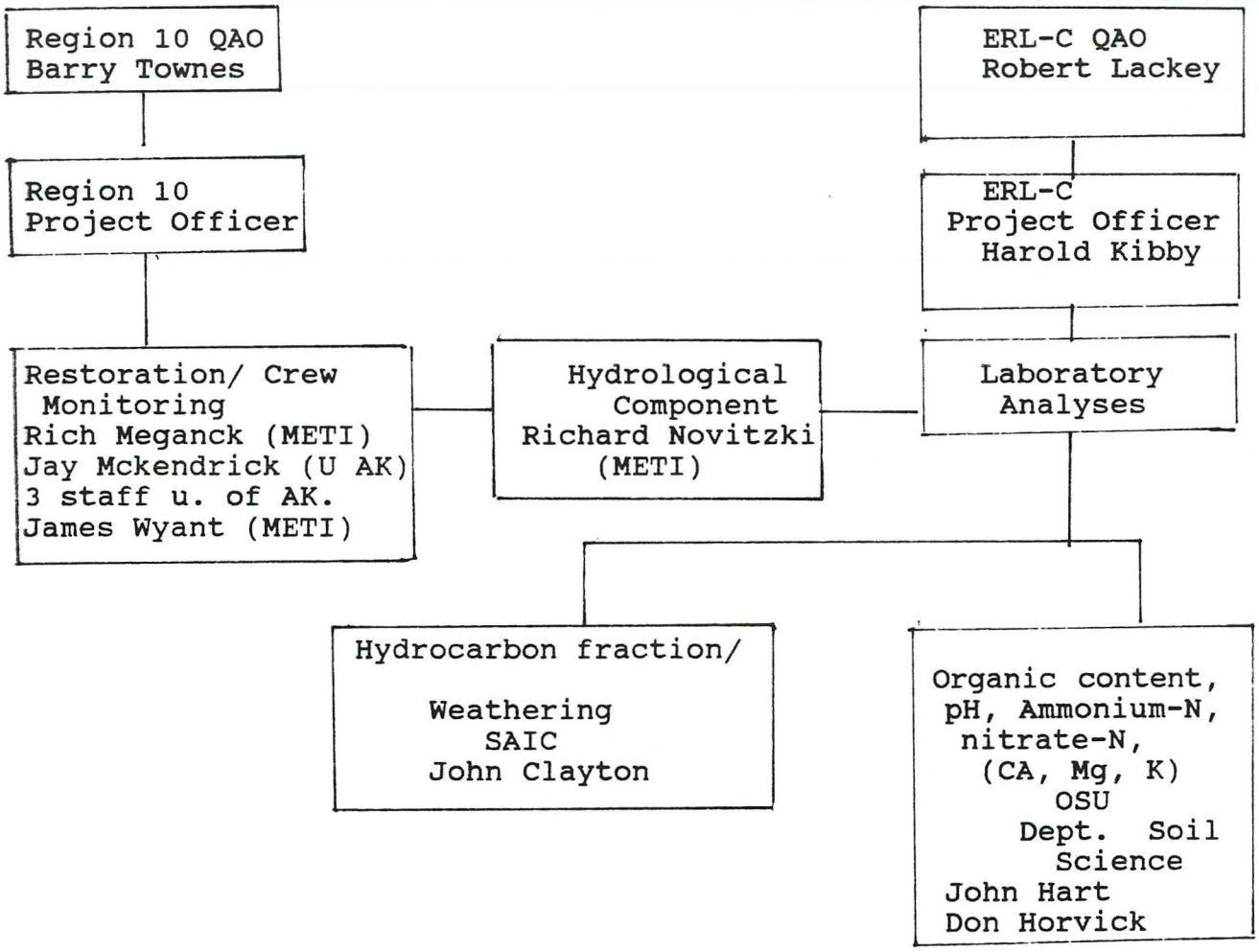

Table 4. Example of Corrective Actions for Operation of Atomic Absoprtion Spectrophotomer

Type of QC Check	Frequency	Precision	Accuracy	Corrective Action
Blank	At beginning of calibration or recalibration		0.00	Check concentration of purified water. Check instrument operating conditions.
Calibration standards	At beginning of each batch analysis		$r^2 \geq 0.98$	Rezero and recalibrate, verify calibration standard values. Check instrument operating conditions. Change O-ring on nebulizer. Replace lamp.
Low concentration QCCS	2X minimum in batch	5%	98% recovery	Reanalyze 2nd duplicate, rezero and recalibrate.
High concentration QCCS	2X minimum in batch	5%	98% recovery	Reanalyze 2nd duplicate, rezero and recalibrate.
Sample duplicates	1 in every 15 routine samples	7%		Reanalyze 2nd duplicate. Reanalyze last 15 samples.
NBS standard*	2X in every batch	5%	1% from certified value	Reanalyze 2nd NBS sample. Rezero and recalibrate.

QC Quality Control
QCCS Quality Control Check Sample
NBS National Bureau of Standards
* or other certified reference material

Figure 2. Agreement to Comply

My signature below indicates that:

1. I have read the QA project plan for the project "Feasibility of Restoring the Bay of Isles and Tonsina Bay in Prince William Sound and the Gulf of Alaska".

2. I have read the QA procedures that are unique to my project activities (Sections 6, 7, 8, 9, 10, 11, 13, 14,15, 16, 17).

3. I accept the responsibility of adhering to the procedures outlined in this QA document.

Signature

2/28/91

Date
