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MARINE POLLUTION MONITORING: STRATEGIES FOR A NATIONAL PROGRAM

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RECOMMENDATIONS TO NOAA

(1) We recommend that a national marine monitoring program for chemical pollution be established. The entry to this program should be based upon the procedures and strategies recommended in this volume.

(2) We recommend that a technical group be established within NOAA to plan and implement a national marine monitoring program for chemical pollution.

(3) We recommend that an advisory panel of scientists from university, government, and private laboratories be established to recommend policies with respect to NOAA monitoring endeavors. The panel should meet at least once a year.

(4) We recommend that NOAA periodically convene workshops of marine scientists to examine the monitoring data, to identify new contaminants and new areas to be monitored, to evaluate collection and analytical techniques, and to assess monitoring strategies.

PREFACE

There are many compelling reasons to initiate a systematic and continuing program of monitoring contaminants being introduced into the marine environment. They pose possible hazards to man's health through ingestion of food products of the sea. There are also potential losses or restricted uses of marine resources. Effects of unpredictable alterations of ecosystems haunt the scientific community. Although these pressing concerns are well recognized, no U. S. governmental agency has yet taken the lead to establish monitoring programs for the already identified contaminants of concern, the halogenated hydrocarbons, petroleum, and heavy metals previously considered in the Brookhaven Report. Twenty-six scientists assembled at Santa Catalina Marine Station under NOAA sponsorship to develop strategies for initiation of monitoring programs for chemical pollutants in the marine environment (artificially produced radioactive species were excluded because they were previously discussed in the RIME report, among others).

In the recent past, there have been rational and effective monitoring programs that have ensured restrictions on releases of materials to the environment. These programs have clearly provided patterns for this present effort. Containment of potentially dangerous levels of radioactive wastes has been effected under the banner of public health -- minimizing the human body burden of ionizing radiation from artificially produced radionuclides as a result of the consumption of food or direct environmental exposure. Restrictions on the use of DDT have resulted from undesirable alterations of the structures of natural communities detected by surveillances of the communities and chemical analyses of their members. Monitoring of reproductive failures and of deaths of non-target organisms through uptake of this biocide has formed a basis for policies limiting its use. Finally, damage to non-living resources,

such as the soiling of beaches or waters by petroleum and its products or the losses of recreational areas through contamination by waste outfalls, has been recognized by appropriate monitoring operations.

In discussions among the participants in this workshop, several concepts emerged to guide the implementation of the recommendations detailed in the following chapters. First of all, every effort should be made to maintain the highest standards of quality during collection and analysis of the samples. Many analyses require highly sophisticated procedures involving expensive instrumentation. The contaminants to be assayed occur at very low concentrations. It is, therefore, easy to contaminate samples, making it difficult (and often time consuming) to get accurate results. For these reasons, the participants recommend establishment of a few analytical facilities, with well-trained scientific personnel who exercise great care and precautions in the collection of samples and in their analyses as opposed to a larger number of laboratories with more limited resources and fewer trained personnel.

Also needed is the ability to respond quickly to anomalous events, either natural or man-induced, that cause dramatic environmental changes. Often an event such as a hurricane flood or accidental petroleum discharge can provide valuable information, which otherwise would be most difficult to come by, on how natural systems respond to impacts. The participants urge that funds be set aside to investigate rapidly and in detail acute disturbances to the marine environment. The sampling and analytical resources of a continuing monitoring program can be applied effectively to such investigations, although some techniques may require modification.

Interactions among government agencies, the academic community and other research laboratories in the past have been most rewarding to all concerned; the participants recommend such interactions be encouraged with the agency (or agencies)

responsible for the monitoring program. There is a clear need for rapid dissemination of monitoring data to the research workers; conversely there is an equal requirement of feedback from the research laboratory to the monitoring program. In addition, the activities of the monitoring program should be coordinated with those of other agencies in order to avoid duplication of effort.

Sponsorship of research, workshops and students by the responsible agency provides a basis for support and involvement of their colleagues in the monitoring program. The participants fully recognize that many important concepts and discoveries in environmental science have evolved from routine surveillance operations, such as those involved with monitoring radioactivity. Information exchanges within the entire environmental scientific community regarding contamination of our surroundings is vital and promises substantial scientific rewards from future monitoring activities.

Edward D. Goldberg
Conference Convener
October 30, 1972

(A) SAMPLING MARINE WATERS

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Introduction

The principal goals of the seawater sampling program are (1) to provide a long-term accounting of the accumulation of chemical contaminants in the marine environment and (2) to obtain some clues to understanding the pathways by which the contaminants are distributed, both before and after they are deposited in the ocean. The program will not serve to give warning of immediate public health hazards; biological indicators will be more useful for that.

To attain these goals the sampling program should take account of: (1) The method of introduction into the ocean (atmospheric fallout, coastal runoff, or man's activities on the high seas), (2) The character of the introduction (continuously, as in industrial discharges; seasonally, as in the spring run-off; or occasionally, as with an oil spill), and (3) The mechanism of transport, both horizontal and vertical, within the marine environment (current systems, biological activity, vertical diffusion, sinking of particulate matter or dispersion as a surface film).

At present there is not enough information to be sure how any of these factors may be related to the distribution of any pollutant; the effects are surely different for each of the three pollutants under consideration. However, baseline studies[1] have provided some indication of the kinds of answers that will be forthcoming, and we therefore operate on the following assumptions:

Atmospheric and coastal processes are both important as sources. Halogenated hydrocarbons are introduced into

the ocean principally from the atmosphere, heavy metals (except for lead) derive principally from coastal runoff, and petroleum has major input from both.[2] Petroleum can also be introduced in significant quantities directly from ships at sea, accidentally or as part of normal operation.

The principal sources of both atmospheric and waterborne pollutants are in the northern hemisphere and exchanges with the southern hemisphere are of secondary importance.

Concentrations in coastal waters are higher and more variable, in both space and time, than those in the high seas. This is borne out by available data for heavy metals and petroleum,[2] but may not be true for the halogenated hydrocarbons (Harvey, personal communication).

Horizontal advection is the principal agent of transport within the marine environment; therefore, concentrations are higher at the sea surface and in the mixed layer than below the thermocline. Exceptions may be particulate material that might sink rapidly to the bottom and vertical transfer by organisms.

Oil spills are the only cataclysmic events that will be reflected in major short-term local changes in concentration outside the estuaries.

Biological sampling programs should be related, where practical, to both the physical characteristics and the pollution levels of the water masses where these samples are taken.

Available sampling techniques require the facilities of a research ship with laboratory space and hydrographic winch, and even the surface sampling must be done while the ship is hove to on station.

Guidelines

The sampling program outlined below is based on the assumptions just listed, but it should also serve to test

them, and some modification will be required as new information and techniques become available.

We recommend:

Widely scattered annual observations in the surface layers in the principal oceanographic regimes of the northern hemisphere, with emphasis on the western Atlantic and the eastern Pacific and with at least one deep series in each ocean and in the Gulf of Mexico.

More closely spaced seasonal observations on the continental shelves, with equal emphasis on the West Coast, the Gulf Coast and the Eastern seaboard. The locations should be (1) off estuaries with important pollution sources and (2) along the axis of prevailing coastwise flow.

The capability to begin sampling on the high seas within a few days of a serious oil spill.

Seawater samples to be taken routinely with all biological samples.

Measurement of temperature and salinity at all sampling sites and depths.

In addition to these fundamental sources of data, we would hope the program could include:

Annual observations (perhaps through international agreements) in the marginal seas of Europe and Eastern Asia.

Occasional sampling (every 3 - 5 years) in midoceanic regions of the southern hemisphere, including the Antarctic.

North-south transects, every 5 - 10 years, in the North Pacific and North Atlantic, similar to the Geosecs program, to tie the annual observations to the general circulation patterns.

Sampling Sites

A. Atlantic

1. Coastal: Quarterly observations (February, May, August, November). Surface only in February, surface and below thermocline in other seasons.

Top priority

- Scotian shelf
- Nantucket shoals
- New York Bight
- Off Norfolk
- Off Savannah

> halfway to 100-fm. curve

Lower priority

- Gulf of St. Lawrence
- Gulf of Maine
- Georges Bank
- Hudson Canyon (@ 50-fm curve)

2. Open ocean: annual observations (season unimportant except where noted).

Top priority

- Labrador Sea - ocean station Bravo (56°30'N, 51°00'W), winter station, samples at surface, 300 m, 1000 m.
- East of Grand Banks - ocean station Delta (44° N, 41°W), winter station, samples at surface, 300 m, 1000 m.
- Sargasso Sea - Bermuda, samples at surface, 300 m, 1000 m, 2500 m.
- Slope Water - ocean station Hotel (38°N, 71°W) samples at surface, 300 m, 1000 m.
- Trade winds, 15°N, 50°W, samples at surface, 300 m, 800 m (salinity minimum) 2500 m (North Atlantic Deep Water), 5000 m (Antarctic Bottom Water).

Lower priority

- Salinity maximum - ocean station Echo (35°N, 48°W), samples at surface, 300 m.
- West of England (54°N, 20°W) samples at surface, 300 m.
- West of Gibralter (36°N, 15°W), samples at surface, 500 m.

B. Gulf of Mexico and Caribbean Sea

These small, relatively isolated portions of the Atlantic Ocean are considered separately because of the strong influence of land on their waters and circulation. The largest part of the drainage from the United States flows into the Gulf of Mexico.

1. Shelf: Quarterly observations (February, May, August, November). Surface only in February, surface and below thermocline in other seasons.

   Top priority

   - Northern Gulf east of De Soto Canyon, near 29.5°N, 87°W.
   - Off Mississippi River, roughly 28.5°N, 89°W, (quarterly samples at a single spot are very probably inadequate.)
   - Off Louisiana coast west of the Mississippi where there are many oil production platforms.
   - Northern Gulf west of Galveston Bay, near 28°N, 96°W, where rainfall is relatively low and evaporation high.

   Lower priority

   - Upwelling region near Cape Catoche, roughly 21.5°N, 87°W.
   - Off Magdalena River, near 11°N, 76°W.
   - Off Lake Maracaibo, near 13°N, 69°W.

2. Open ocean (annual sampling at surface and in thermocline)

   - Interior of the Gulf Loop Current (Southeastern gulf roughly 24°N, 85.5°W) supplied mainly by inflow through Yucatan Strait.
   - Western Gulf anticyclonic region (24°N, 93°W)
   - High rainfall region north of Isthmus of Panama (11°N, 81°W)
   - St. Vincent Passage where very large inflow into Caribbean exists and where rainfall is relatively low (included with open ocean because of its importance in that realm)

Samples below the thermocline (annual)
at the Western Gulf anticyclonic region station in depths of the oxygen minimum (10°C), salinity minimum (6.5°C), and deep water below the temperature minimum (4.25°C roughly).

C. Pacific

1. Coastal areas (quarterly samples at surface and in thermocline except in winter, when surface sample will suffice.)
   - near Valdez, Alaska
   - near Puget Sound (this and previous location because of their relation to TAPS)
   - near the Columbia River plume
   - off Point Conception
   - near Los Angeles outfall
   (Comparable locations off the coast of Japan may be desirable.)

2. Open ocean (annual samples at surface and in thermocline)
   - Stations along 155°W
     at 50° N (subarctic water) (or possibly station Papa)
     at 40° N (transition water)
     at 25° N (central water)
     at 10° N (Equatorial high rainfall zone)
   - Station November (30°N, 140°W) as a control for coastal sampling

   Water samples below thermocline (annual)
   - at Station November in depths of the salinity minimum, the oxygen minimum and the deep water.

References

1. Baseline studies of Pollutants in the Marine Environment and Research Recommendations: The IDOE Baseline Conference, May 24-26, 1972, New York, 1972.

2. Man's Impact on Terrestrial and Oceanic Ecosystems. W. H. Matthews, F. E. Smith and E. D. Goldberg, editors. The MIT Press 1971.

(B) SAMPLING ESTUARIES

Contributors: Harry H. Carter
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Introduction

Estuaries are important in themselves as parts of the marine environment and may be important pathways for the introduction of certain of the heavy metals, halogenated hydrocarbons, and petroleum products to the sea. An estuarine monitoring program for these three classes of pollutants should: (1) serve as a basis for assessing any immediate public health hazards, (2) document trends in water quality within the estuarine zone, and (3) provide data for assessing the relative importance of these regions as inputs to the sea.

For initiation of the program we recommend selection of a relatively small number of major estuarine areas that have great socio-economic value and pollution potential and which encompass a variety of estuarine types.

As the ability to monitor satisfactorily is improved and demonstrated, the program should be expanded to include other estuarine regions. Initially we recommend that the following estuaries be monitored:

Prince William Sound	Galveston Bay	Chesapeake Bay
Puget Sound	Mobile Bay	Delaware Bay
San Francisco Bay		Long Island Sound

Immediate Health Hazards

Public health hazards from heavy metals, halogenated hydrocarbons, and petroleum arise principally through human

consumption of contaminated shellfish and finfish. Accordingly, these substances should be monitored in the organisms themselves. The organisms to be monitored and the monitoring frequency are listed in Chapter 2. We recommend that shellfish from different salinity regimes in a given estuary should be sampled since the uptake of certain heavy metals is apparently salinity dependent.

Water Quality Trends

A major objective of the monitoring program is to establish persistent trends in water quality; trends which may be useful in assessing man's impact on the estuarine environment. This must be a relatively long-term objective since marked temporal variations of the concentrations of many naturally occurring and man-made substances occur in estuaries. For this purpose we recommend monitoring of the estuarine waters, sediments, and biota for heavy metals, halogenated hydrocarbons, and petroleum.

Water sampling. We recommend establishment of standard stations in the estuarine systems for sampling of pollutants in dissolved and/or suspended particulate form. The primary natural pathways for the introduction of pollutants into the estuarine environment are rivers. We recommend that the major rivers flowing into the aforementioned estuarine systems be sampled weekly in their lower reaches, but well above the upstream limit of measurable sea salt. Rivers are covered in Chapter 3. The other important pathway for the introduction of pollutants is through municipal and industrial outfalls. These should be sampled at the source -- in the pipe. Sampling of outfalls is covered in Chapter 3. Spills may also contribute relatively large amounts of contaminants in short periods of time, and these inputs should be determined. Effective monitoring of catastrophic events can be achieved only through intermittent intensive sampling programs which can be instituted rapidly,

and not through routine monitoring. Of more direct importance to the proposals in this chapter is the establishment of one or more mid-estuary stations, depending upon the complexity of the particular system. These stations should be established well away from the influence of local inputs. They should be occupied at approximately monthly intervals. Samples should be taken from near the surface, mid-depth, and approximately two m from the bottom. Care must be taken to occupy these stations at a consistent phase of the tide, preferably slack water, in order to minimize difficulties of interpretation. Measurements of temperature, salinity, and pH should be made concurrently with the foregoing.

Sediment sampling. Sediment samples should be collected at a number of locations within each estuary. Because of the association of each of these contaminants with fine-grained sediment, these stations should be selected in areas where fine-grained materials are accumulating. The station locations should encompass the normal range of salinities, and the sampling should be repeated at approximately 2 - 3 year intervals. We also recommend that short cores be taken at these stations and that analyses be made to determine the vertical distributions of heavy metals, halogenated hydrocarbons, and petroleum in the sediments. These data may be useful in assessing trends in man's impact on the estuarine environment.

Biota sampling. Information on long term water quality trends may perhaps also be inferred from the sampling of the biota for public health hazards if the levels of contaminants are determined with sufficient precision and accuracy and the data archived.

Inputs to Ocean

To assess the relative importance of the inputs to the sea of heavy metals, halogenated hydrocarbons, and petroleum from estuaries, we recommend that water samples be collected on a monthly basis in the major outlets to the sea of each of the estuaries listed above. Samples should be collected near the surface, at mid-depth, and at 2 m above the bottom.

Sampling should be done at the end of ebb. Concurrent measurements of temperature, salinity, and pH should be made.

2

SAMPLING MARINE ORGANISMS

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Introduction

The purposes in monitoring marine organisms are threefold: (1) Many of them are of immediate importance to man as food, and hence the concentrations of pollutants in their bodies may present health hazards. (2) Because marine organisms concentrate most pollutants they are useful as indicators of the spatial and temporal distribution of pollutants. Further, (3) it seems likely that the biosphere represents an important reservoir and transport medium for pollutants and it is therefore of great importance in the attempt to determine mass balances of pollutants in the world system. Monitoring cannot provide all of the kinds of information required for a complete understanding of the processes and events involved in the injection and dispersal of pollutants; however, the spatial and temporal data that can be generated from well designed monitoring programs are essential to this understanding.

We propose a program of monitoring the coastal and oceanic regions of the North Atlantic, North Pacific and Gulf of Mexico and have chosen what we believe to be a reasonable spectrum of species. The organisms were chosen on the basis of several criteria; importance to man, ease of sampling, ecological "importance", and biochemical, physiological and behavioral diversity. The sampling sites were chosen to be representative of major coastal and oceanic habitats each of which differs in ecosystem structure and function.

Although we feel a monitoring program to be of great potential value, there are serious problems involved in the interpretation of sample data. Because of the patchiness of both spatial and temporal abundances and of variations in physiological and behavioral states and of varying age frequency distributions we expect large within habitat variances of all measured variables. It is essential, therefore, to conduct pre-monitoring sampling studies at each of the sites and for each of the species of organisms and pollutants. The essential purpose of such studies would be to provide some preliminary information on the relationship of the samples to the universe being sampled. We advise a series of replicate samples to be taken of each species at each site to be measured for each of the pollutants. Variance estimates could thus be derived which would be of some use in the interpretation of subsequent samples taken during the monitoring program.

Coastal Zone

Organisms have been recommended that when sampled will give a cross-section of trophic levels where different degrees of pollutant accumulation may occur. Species have been selected which feed in the water column and on the bottom, which is where all pollutants ultimately reside. Groups have been chosen because of possible differences in pathways due to physiologies and biochemistries. Filter feeders, for example, are much more closely associated with the water, and their mucus absorbs material directly from the water much more readily than feeding mechanisms of particulate feeders. Anadromous fishes have been recommended because they migrate into and out of areas which may be more highly polluted.

A minimum number of sampling sites is recommended. The basis for the selection of these sites is to have one contaminated and one "clean" location on each coast.

Species recommended for sampling are enumerated in Table 2.1. At the bottom of the food chain the molluscs *Mytilis* and *Rangia* are recommended. These filter feeding organisms have a wider distribution (*Mytilis* on the east and west coasts, *Rangia* on the Gulf coast) than do commercial oysters but occupy much the same position in the food chain.

Herring, menhaden, and anchovy are widely distributed plankton feeding fishes. They are caught commercially and are animals with a rather high oil content which may be of advantage in monitoring halogenated hydrocarbons and petroleum.

Squid - *Loligo* - is a widely distributed carnivore on all coasts which is fed upon by commercial fishes and which also are fished commercially.

Yellow tail flounder and dover sole are flat fish which feed on the bottom and are fished commercially. Blue crab and Dungeness crab are also bottom feeding organisms which are probably more omnivorous than the flatfish. They are important commercial species.

Striped bass are top carnivores which have a rather general distribution and are heavily fished by sports fishermen. They represent an anadromous fish which may be substituted for in the North Pacific and Alaskan Coasts by the Silver salmon.

Sampling Locations

Alaska - coastal sampling in the area of Valdez-Pacific Coast of the continental U. S., the area of the "Los Angeles bight", is recommended as the highly contaminated area; a location north of the Columbia River outflow is recommended for the clean site. On the Gulf coast it is suggested that the sampling take place in the area of the Mississippi outflow for the contaminated area. The Flower Garden Banks off Texas-Louisiana are put forth as a relatively clean area.

On the Atlantic coast three sites are recommended: off the Savannah River and in the "New York bight" for contaminated areas and along the North coast, north of Boston, for a relatively clean area.

Oceanic Zone

Organisms are selected to give as wide as possible a selection across trophic levels Table 2.2 However, because of contamination problems associated with sampling phytoplankton, the lowest level in this food chain is the zooplankton. These primary filter feeding organisms are in close contact with the water and should give an early indication of the appearance of pollutants. Notes should be made of at least the general composition of the collection, e.g., the proportions of salps vs. Crustacea.

Myctophids and squid are selected as important components of the food web which are fed upon by the important oceanic commercial species. They may also give some comparison with animals in the coastal zone.

The high order carnivores recommended include three species in order to give coverage over the oceanic zones - sub-arctic, transition, central, and equatorial. These three are: pink salmon, albacore, and yellow fin tuna. In the Atlantic Ocean the bluefin tuna is substituted for the salmon and albacore.

Another high order carnivore, the dolphin fish, is recommended because of its wide distribution in the Pacific, Atlantic, Gulf, and Caribbean. It is easily caught and will give an excellent area-to-area comparison.

Sampling locations in the Pacific Ocean are along the Kodiak-Hawaii transect in each of the zones to be sampled. Some excursions south of Hawaii may be necessary to sample the tropical zone. Sampling in the open Atlantic, Gulf of Mexico and Caribbean should correspond to the suggestions made in chapter 1(A).

Since the zooplankton - myctopid-oceanic squid samples are likely to be mixtures of species subsamples, these should be preserved for identification.

Table 2.1: Coastal species selected for monitoring

Type*	EAST COAST Common Name	EAST COAST Scientific Name	GULF COAST Common Name	GULF COAST Scientific Name	WEST COAST Common Name	WEST COAST Scientific Name
1.	herring	*Clupea harengus*	menhaden	*Brevoortia tyrannus*	anchovy	*Engraulis mordax*
2.	yellowtail flounder	*Limanda ferruginea*	Southern flounder	*Paralichys lethostigma*	Dover sole	*Microstomus pacificus*
3.	striped bass	*Roccus saxatilis*	red snapper	*Lutjanus campechanus*	striped bass silver salmon	*Roccus saxatilis* *Oncorhynchus kisutch*
4.	mussels	*Mytilus edulis*	rangia	*Rangia cuneata*	mussels	*Mytilus edulis* *M. californianus*
5.	squid	*Loligo pealei*	squid	*Loligo pealei*	squid	*Loligo opalescens*
6.	blue crab	*Callinectes sapidus*	blue crab	*Callinectes sapidus*	dungeness crab	*Cancer magister*

* Reasons for selection:

1. plankton feeding fishes of wide range, high in lipid content and of commercial importance
2. benthic feeding fishes of wide range and of commercial importance
3. top carnivores: red snapper selected because of commercial importance; striped bass selected because they are anadromous and likely to occur in areas with high pollution; silver salmon selected because of commercial importance and wide range
4. sessile filter feeders with wide distribution
5. fed upon by many important commercial species and also of commercial importance themselves
6. representative crustaceans of commercial importance and wide range.

Table 2.2: Open ocean species selected for monitoring

Type*	Common Name	Scientific Name
1.	yellowfin tuna	*Thunnus albacares*
2.	albacore tuna	*Thunnus alalunga*
3.	pink salmon**	*Onchorhynchus gorbuscha*
4.	dolphin (mahi mahi)	*Coryphaena hippurus*
5.	macrozooplankton	
6.	myctophids	
7.	squid	*Ommastrephes* spp.

* Reasons for selection

Types 1-3 were selected because of their tremendous commercial importance and because they are wide-ranging, top predators. Type 4, another top predator, was selected as it is found in the Central Pacific gyres not frequented by types 1-3. Types 5-7 comprise important components of the food web supporting the top predators.

** Bluefin tuna (*Thunnus thynnus*) should be substituted for salmon in Atlantic Ocean.

3

SAMPLING THE TRANSPORT PATHS

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Introduction

The avenues of transport of possible pollutants to the sea can be divided into four categories: (1) the atmospheric paths, (2) streams, (3) direct injection from outfalls, and (4) introduction by ships. A proper monitoring program should minimally assess the flux of pollutants at critical points in the marine realm ascribable to each of the pathways. In order to provide the most reliable estimate of pollutant flux, an attempt must be made to assess what fraction of the locally measured flux is ascribable to natural or ambient origins. For PCB's only man-made sources need be considered, but for the heavy metals and petroleum unless the natural flux is known in each, the total flux may be an incorrect estimation of the effects of man.

In the initial stages of a monitoring program it is evident that a number of stations must be set up at apparently critical points along the several pathways indicated above. The measurements made at these stations on the appropriate chemical parameter must then be related to the various types of contributions made to that point in feedback fashion. If the monitoring station does not record events clearly sensed at other locales in the system, the monitoring network should be redesigned to accommodate the new information.

Sampling Plan

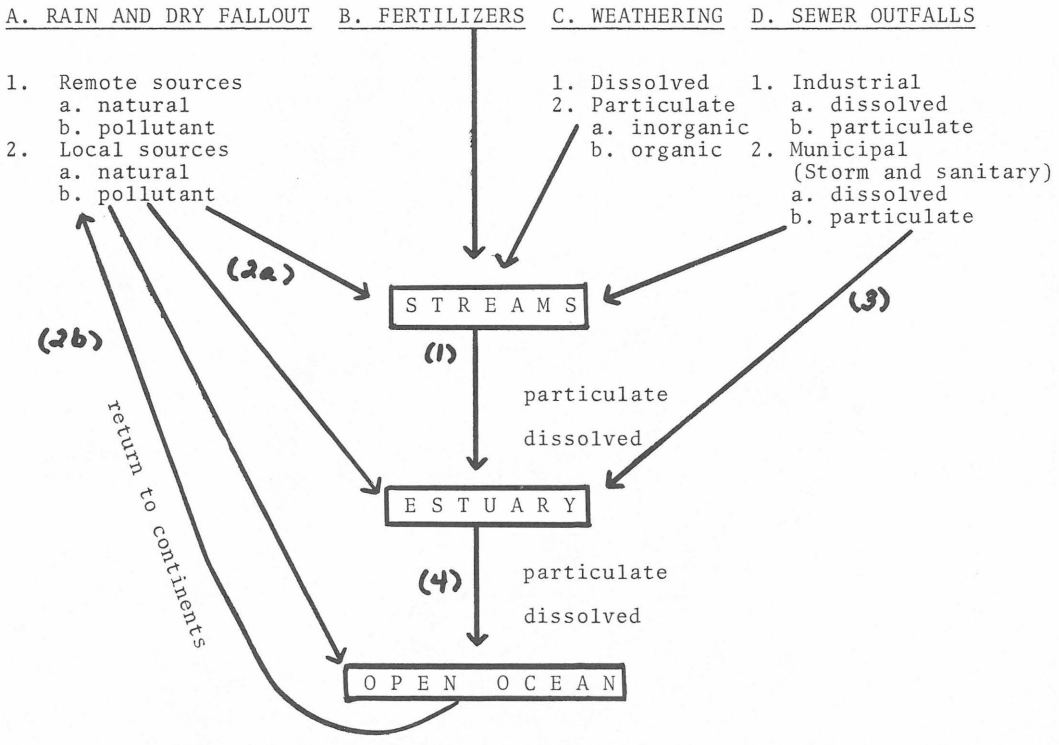
Principal flow paths for materials carried to the oceans will be sampled. The circulation routes indicated by arrows in Figure 3.1 will be monitored at locations indicated by number (1) to (4) and described as follows.

(1) The particulate and dissolved flux of the critical elements supplied by streams should be measured at the closest point of injection into coastal waters. A working list of the actual streams to be sampled are evaluated in a later section.

(2) The atmospheric flux of particles through precipitation and dry fall-out should be measured both for the incoming contribution to the continents (ultimately to be carried by streams) and the outgoing flux to estuarine and open ocean areas. Some monitoring sites are given in the following section.

(3) Sewer outfalls debouching directly into the coastal zone should be monitored as described below. In the initial monitoring set-up it will be assumed that the stream analysis will integrate all sources along the stream including sewer outfalls.

(4) The flux outward from the estuary to the open ocean will have to be monitored where identifiable, at exits of large estuarine mixing basins. This is discussed in detail in the section on estuaries.


A. RAIN AND DRY FALLOUT
1. Remote sources
a. natural
b. pollutant
2. Local sources
a. natural
b. pollutant
B. FERTILIZERS
C. WEATHERING
1. Dissolved
2. Particulate
a. inorganic
b. organic
D. SEWER OUTFALLS
1. Industrial
a. dissolved
b. particulate
2. Municipal
(Storm and sanitary)
a. dissolved
b. particulate
(2a)
(2b)
(3)
STREAMS
(1)
particulate
dissolved
ESTUARY
(4)
particulate
dissolved
return to continents
OPEN OCEAN

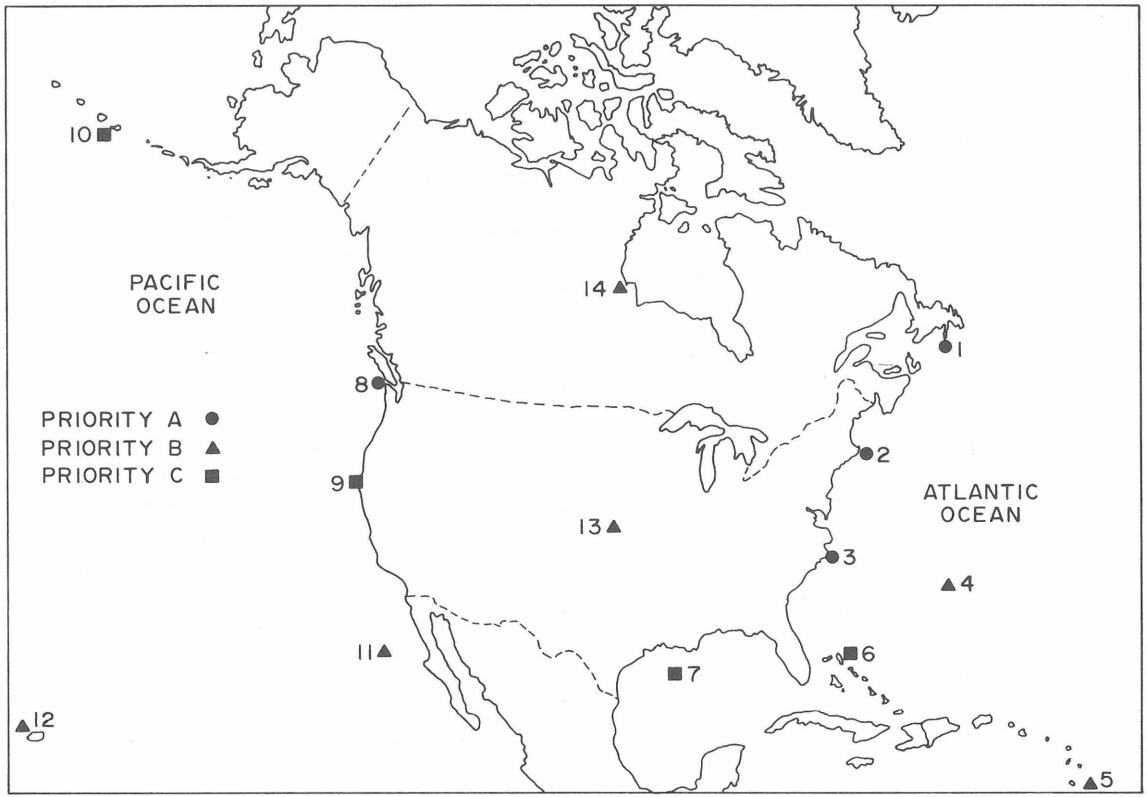


Figure 3.1 – Atmospheric sampling sites

Atmospheric Transport

Sampling Sites

The atmosphere is a major transport path for most pollutants found in open ocean regions. Any atmospheric monitoring program for pollutants from North America should be designed to give information on the flux of atmospheric material to open ocean regions and the rate of deposition of this material on the sea surface. The primary transport to the open ocean occurs on the prevailing westerlies north of 30°N on the east coast and the northeast trade south of 30°N on the west coast. Information should also be obtained on the influx of the various pollutants to North America on the prevailing westerlies north of 30°N on the west coast and the northeast trades south of 30°N on the east coast.

Recommended atmospheric sampling sites are divided into three groups: Atlantic, Pacific, and Continental. Their locations are given below and in Figure 3.1. Priorities for atmospheric sampling sites are defined as follows:

Priority (A) - Highest priority, site should definitely be in an air sampling program.

Priority (B) - High priority, site should be in an air sampling program if possible.

Priority (C) - Low priority, desirable for site to be in an air sampling program but not critically important.

A. Atlantic

A maximum of 7 stations are recommended in the Atlantic sector. The locations of these stations are designed to give maximum information on a) the transport of atmospheric pollutants from the North American continent to open ocean regions north of 30°N and b) the influx of airborne substances to North America south of 30°N.

(1) Site 1. (Priority A) - Tower site on the coast on the Avalon Peninsula, Newfoundland, preferably on Cape Pine or possible Cape Race. During

much of the year (especially spring, summer, and fall) Newfoundland lies almost directly downwind from the major pollution areas in the northeastern United States. It is thus ideally located for monitoring the quantities of atmospheric pollutants capable of reaching well beyond the continental shelf. Cape Sable, Nova Scotia, which is several hundred miles closer to the northeastern United States, is an alternate site.

(2) Site 2. (Priority A) - Nantucket Light Ship or a tower on the west coast of Nantucket Island. This location, also downwind from major northeastern U. S. pollution sources, will provide information on atmospheric pollutant transport over coastal waters and will be useful for comparison with the Newfoundland station.

(3) Site 3. (Priority A) - Diamond Shoal Tower, Cape Hatteras, North Carolina or Frying Pan Tower, Cape Fear, South Carolina. This site will provide information on the atmospheric transport of pollutants off the coast of the mid-Atlantic states in a region with few major pollution sources.

(4) Site 4. (Priority B) - Bermuda Air Sampling Tower operated by the University of Rhode Island as part of the International Decade of Ocean Exploration. The Bermuda station, located approximately 750 miles off the east coast of the U. S. at 32°N, is under the influence of surface westerlies with trajectories over North America during the winter months but predominantly southeasterlies from the eastern Atlantic during the summer. This station thus offers marked seasonal contrasts in air masses to be sampled.

(5) Site 5. (Priority B) - Barbados Air Sampling Tower operated by the University of Miami. This

tower, in the northeast tradewinds, offers a stie for obtaining background data on the atmospheric input to the Caribbean area of pollutants which have been transported across the Atlantic Ocean.

(6) Site 6. (Priority C) - Tower site on the east coast of one of the Bahama Islands, e.g. Great Abaco or Eleuthera. This location, at 25° - 27°N, is generally under the influence of prevailing easterlies and would give information on the atmospheric input of the substances of interest to the southern U.S.

(7) Site 7. (Priority C) - A site on one of the offshore oil rigs in the Gulf of Mexico. This site would give information on the atmospheric transport of pollutants to this relatively closed body of water.

B. Pacific

A maximum of 5 stations are recommended in the Pacific sector. The location of these stations is designed to give maximum information on a) the transport of atmospheric pollutants from North America on the northeast trades south of 30°-35°N and b) the influx of airborne substances to the North American continent north of 30°-35°N.

(1) Site 8. (Priority A) - Tower site on Tatoosh Island, Washington. This location would give background data on atmospheric pollutant input to North America via long range transport from Asia.

(2) Site 9. (Priority C) - Tower site at Cape Mendocino, California. This site would also give information on pollutant input to the continent via marine air, but at a lower latitude than Tatoosh Island.

(3) Site 10. (Priority C) - Tower site on Adak or Atka in the Aleutian Islands. This site would give background information from a mid-

oceanic location on atmospheric transport of pollutants across the Pacific from Asia.

(4) Site 11. (Priority B) - Tower site on Guadalupe Island, Mexico, located at 29°N, 118° 30'W. This site, located approximately 300 miles south (and downwind during much of the year) of Los Angeles, would provide information on atmospheric pollutant transport to the ocean off southern California. An alternate possibility to this site would be one of the islands off Los Angeles, e.g. San Clemente or San Nicolas.

(5) Site 12. (Priority B) - Hawaii Air Sampling Tower located on northeastern Oahu, operated by the University of Hawaii. This location is ideal for monitoring atmospheric pollutants transported from the west coast of North America on the prevailing north-east trades. An alternate site in Hawaii would be the NOAA Mauna Loa Observatory, a high level site over 11,000 ft. above sea level on Hawaii Island.

C. Continental

Only 2 stations are recommended in the continental sector. It is assumed that any samples or data needed from urban areas can be obtained through the EPA's National Air Sampling Network.

(1) Site 13. (Priority B). Site in a rural area in the central United States, possibly Kansas or Nebraska. Data on the pollutants to be measured in this program are virtually nonexistent in non-urban continental areas and will be of considerable value in trying to assess medium to long range atmospheric transport.

(2) Site 14. (Priority B) - Site at Churchill, Manitoba, on Hudson's Bay. Rationale is the same as for Site 13.

Sample Collection

A. General

Extreme care must be taken during the collection and handling of atmospheric particulate and gas samples, especially at the more remote sites. Atmospheric concentrations of all the substances of interest will be so low in these areas that even the slightest contamination will render a sample useless. Aside from the instrumental safeguards to minimize contamination, described below, personnel collecting the samples must be thoroughly familiar with precautions to be taken during sample collection and handling and must be aware of the many sources of contamination that can arise. The hands, hair, and clothes of personnel should be covered when handling these samples, and maximum use should be made of laminar flow clean stations. Samples at coastal sites should be collected from towers sufficiently high, perhaps 20 to 30 meters, to avoid local sea spray as much as possible. When ships or off-shore towers or rigs are used as collection platforms, an additional tower should be constructed to elevate the collection apparatus several meters above the deck of the primary platform to avoid local contamination problems.

B. Meteorological and Ancillary Equipment

(1) Directional Air Sampling Controllers and Wind Recorders

In any atmospheric monitoring program it is critical that as much as possible be known about the past history of the air sampled. It is recommended that sampling equipment be controlled by wind direction. Equipment for this purpose is presently commercially available and allows one to operate collection apparatus when the wind is blowing from any predetermined sector. This type

of control is extremely important in attempting to determine atmospheric fluxes into or out of a particular area, and can also serve to prevent local contamination of samples. Permanent records of wind speed and direction at these sites should be maintained.

(2) Condensation Nucleus Counters
It is also recommended that a recording condensation nucleus (CN) counter be located at each site. Pollution particles are excellent condensation nuclei, and monitoring changes in CN over a period of years would give information on general air quality trends at the sampling sites. In addition, short term rapid variations in CN counts can alert the investigator to air mass changes and/or possible local contamination of samples. CN counters should be integrated into the directional air sampling central system at remote sites so that collection equipment only operates when the CN count is near background levels for marine air.

(3) If the site is not near a permanent meteorological station, it is recommended that a recording rain gauge be installed.

(4) Synoptic Weather Charts
Maximum use should be made of surface synoptic charts as well as 850 mb, 700 mb, and 500 mb upper air charts to ascertain the past history of the air mass being sampled. Correlation of air trajectories with pollutant concentrations will be a critical part of any meaningful program on atmospheric transport.

C. Particulates

(1) Heavy Metals
Atmospheric particulate samples for heavy metal analysis should be collected on either Whatman 41

or Delbag Microsorban polystyrene filters. The former are recommended due to a low metal blank, low cost, and ease of handling in analysis. Filters should be mounted in plastic filter holders, preferably polyethylene, and the holders should be in plastic shelters to protect them from rain. High volume pumps should be located at least 4-5 meters downwind or below the filter holders to prevent collection of pump produced contamination. Operation of the pumps should be controlled by wind direction and, at remote sites, by CN counts, as described previously. Doors or some other method of protecting the filters automatically when the pumps are not running are also recommended to prevent filter contamination when the collection system is not operating. Filters should be stored in sealed polyethylene containers, preferably in a freezer at -20°C.

(2) Petroleum and Chlorinated Hydrocarbons
Atmospheric particulate samples for hydrocarbon analysis should be collected on Type A glass filters which have been pre-ignited for several hours at 400-500°C. Filters should be mounted in aluminum or stainless steel filter holders housed in metal weather shelters. The pumps should be located several meters from the filter holders to prevent contamination, and collection should be controlled by wind direction, and CN counts. Filters should be stored in metal containers in a freezer at -20°C.

D. Gases

The distribution of atmospheric hydrocarbons, particularly the chlorinated hydrocarbons such as PCBs, DDT, etc., between the gas and particulate phases is presently unknown. Collectors for gaseous hydrocarbons

are presently under development at several laboratories. It is strongly recommended that the gas phase of these substances be monitored in this program.

E. Precipitation and Dry Fallout

The most important information which can be obtained from these monitoring stations is an estimation of the flux of the various atmospheric pollutants to the sea surface. Provisions should be made at the sampling sites for collection of precipitation and dry fallout. Collectors should have a surface area of approximately one square meter. Samples to be analyzed for organic pollutants should be obtained from collectors constructed of stainless steel or aluminum while those to be analyzed for heavy metal should be obtained from collectors constructed of polyethylene. The collection system should be automatically controlled so the rain collector is exposed only during rainfall, and the dry fallout collector is exposed at other times. As with the filter samples neither of the collectors would be exposed unless wind direction and CN counts are appropriate.

F. Glaciers and Snowfields

While not part of a monitoring program per se, glaciers and snowfields can give very valuable information on the current and past deposition rates of the various pollutants. By use of oxygen isotopes and lead-210 geochronology, the age of the ice samples can be ascertained. For this program, coastal glaciers in Alaska, the Yukon, British Columbia, and Washington could be used for data on input rates of pollutants to North America from the prevailing westerlies. Glaciers in Mexico (e.g. Popocatepetl) could be used for data on input rates from the northeast tradewinds.

Sample Duration

In atmospheric sampling, one treads a fine line between sampling too short a period of time for collection of sufficient material to ensure meaningful analyses and sampling

for such a long period of time that variations in meterological conditions obscure significant short-term atmospheric concentration variations. In general, a sampling period of about two days is recommended for particulates, with a similar time probably required for gaseous organics. Use of high volume pumps capable of flow rates of 100-400 m^3/hr will result in samples from 5,000-20,000 cubic meters of air. The proposed sites should be in operation simultaneously for a period of approximately three weeks during each of the four seasons. This would yield an annual collection of a maximum of 45 particulate samples for metal analysis and 45 for organic analysis as well as 45 gas samples for organic analysis at each site. If only the four Priority A stations were operational, this would result in a maximum of 180 samples per year for metal analysis and 360 for organic analysis. If all 14 stations (Priority A, B, and C) were operational, the corresponding maximum number of samples would be 630 and 1260.

Sampling duration for total fallout samples would be variable, depending upon meteorological conditions, but may average 5 to 7 days in duration, resulting in approximately 12-16 samples/year at each station. This would give a maximum of an additional 64 samples per year for metal analysis and the same number for organic analysis at the four Priority A stations or 224 samples per year for metals and organics if all 14 stations are operational. The actual number of samples collected would likely be reduced by approximately 25% from the numbers above due to adverse weather conditions during collection periods.

River Transport

Sampling Criteria and Supplementary Data

An inventory of the contributions of heavy metals, halogenated hydrocarbons or petroleum products by river water to the marine environment requires collection and analysis of samples of water and associated suspended material from

streams that enter the ocean. Samples should be obtained at sites where the river flow rate is measured continuously, sufficiently upstream from the river mouth to be free from the influence of salty water. Samples must be obtained frequently enough to show the extent to which concentrations change with changes in flow, seasonal weather patterns, and other related factors. The analyses must include separate determinations of contents of metal and other materials in solution and in suspension, and sample collection equipment and techniques must be capable of giving an uncontaminated water and sediment mixture in the samples that represent accurately the actual condition in the whole river cross section at the time of collection. The samples must be handled after collection in such a manner as to preserve the initial distribution of metal ions between solute and particulate forms. Analytical techniques must be sufficiently sensitive to detect and measure the low concentrations that generally will be present in solution. Determination of metal content in particulate inorganic or organic material must be preceded by a separation step to bring into solution the metal content of these materials that can reasonably be considered to be available when they are deposited in estuaries and near-shore ocean water. In order to utilize the results of the work most effectively the sampling site should be one for which other organized water quality data are being obtained, particularly sediment concentrations and loads and concentrations of major ions.

Certain ephemeral properties of river water must be measured in the field at the time of sampling if meaningful values are to be obtained. Among the most obvious of such properties are water temperature, dissolved gases, pH, and alkalinity.

Because of the general tendency for many of the metals to be associated with particulate material rather than being in solution, the sampling program must pay special attention to the suspended phase. This may entail a concentrated sampling effort during higher flow stages and utilization

of special equipment, because a very high percentage of the total metal discharge may occur at such times.

Much of the foregoing commentary applies also to sampling for halogenated hydrocarbons and petroleum. Very little development of analytical methods for the petroleum materials in river water has been done, but for halogenated hydrocarbons some highly sophisticated equipment is now available that is capable of determining extremely low concentrations. A substantial volume of data on occurrences of these materials in U.S. river water and sediments is available (Crump-Wiesner, Feltz, and Yates, 1972).

Sampling Sites

The river discharge to the ocean from the conterminous United States, Alaska, and Puerto Rico was recently recalculated by Wilson and Iseri (1969). Flows of all the major streams in the conterminous 48 States are regularly measured and some water quality information is obtained for most of these near their points of discharge to the ocean. Only about 1/4 of the total discharge of Alaskan streams into the ocean is actually measured.

The subjoined list Table 3.1 includes stream gaging and sampling stations for almost all the streams in the conterminous 48 states whose discharge to the ocean was indicated by Wilson and Iseri (1969) to be 200 m^3 sec^{-1} or more, and some smaller streams that may represent large drainage areas or potentially important sources of metals. Information was obtained in part from the U.S. Geological Survey report series "Surface Water Resources of the United States 1961-65", and from a summary by the Office of Water Data Coordination, USGS, (1972) of facts relating to the National Water Data Network. The pertinent information as to nature of records available and most of the records themselves are stored in a computerized data bank, from which the desired material can be recovered. Extensive efforts are made to make data available as soon

after collection as possible.

Drainage areas and discharges in Table 3.1 are those for the sampling or associated flow-measurement site and in some instances additional inflow occurs downstream.

The total discharge for the 47 tabulated stations for the conterminous U.S. represents 74% of the 49,440 m^3 sec^{-1} reported by Wilson and Iseri (1969) as the total runoff to the ocean for this area. Streams discharging into Canada are not included--the major one is the Saint Lawrence River which carries outflow from the Great Lakes amounting to more than 2700 m^3/sec, according to Wilson and Iseri (1969). If it were desired to measure total output from this stream however it should be sampled near its mouth in Canada.

It would appear that sampling at a selected group of the stations listed in Table 3.1 would, by reasonable extrapolation, provide a satisfactory basis for determining the total load of metals contributed to the ocean. From consideration of available data, geologic and cultural factors, and runoff characteristics, a group of 25 stations is designated in Table 3.1 as suitable for the desired purpose. Some further contraction of the number would be possible at the price of a somewhat decreased confidence in the final estimate. The 25 stations still represent about 65% of the total water discharge to the ocean.

Almost half of the total water discharge to the ocean from the 48 states is from the two river systems, the Mississippi and the Columbia, and could be represented by only four of the sampling stations in the list. However, there is an obvious need for considering the geographic distribution of sampling stations as well as total discharge. Reduction of the number below the designated 25 is not desirable for this reason. Substitution of undesignated stations for one or more of those in the list may however be necessary if unforeseen conditions at a particular site make it impractical to conduct operations there.

The four Alaskan stations represent about 15 percent of the discharge to the ocean from Alaska estimated by Wilson and Iseri (1969). A much greater amount of extrapolation is needed to evaluate the discharge of metals from Alaska than in the conterminous 48 States.

Discharge from Hawaii is considered negligible because of the small area and runoff volumes involved.

Almost all the stations in the list are proposed for inclusion in the National Stream Quality Accounting network being activated by USGS. Among data to be obtained in this program will be a quarterly analysis of water and sediment samples for heavy metals. This program also will include analyses for certain halogenated hydrocarbons. Stations being activated in 1973 are designated by footnotes to Table 3.1.

Frequency of sampling and other details of determining heavy metal contents in the NSQA network program may not entirely serve the needs of the IDOE baseline study, but coordination of the work will obviously be desirable to obtain maximum benefit for efforts expended.

For most of these stations there are some existing records of chemical quality, and many sediment concentrations and loads have been measured. The indicated data on minor elements represent dissolved material only and are fragmentary.

Frequency of sampling and elements determined

It is desirable to emphasize again that particular attention needs to be given to the content of metals in the undissolved river loads. Some of this material may consist of metal hydroxide particles, inorganic sediment and adsorbed metal ions, and organic materials living and dead of all types that may contain combined metals. Improper treatment of these materials will introduce very large errors into the estimated total metal contributions, and it is far better to obtain good data at a few points than poor data at many points. If a choice must be made,

this fact is of paramount importance. Some aspects of determinations of trace elements in river water have been discussed by Eichholtz, Galli, and Elston (1966) and by Turekian and Scott (1967).

The frequency with which samples should be collected at the recommended sites should be sufficient to determine concentrations of heavy metals and other materials at a wide range of conditions--high and low flow stages, high and low sediments concentrations, and at different seasons of the year. When the effects of these factors have been appraised, the sampling frequency at the site probably could be decreased and sampling effort concentrated on those times when major amounts of the metals seem most likely to be present. For the first year of operation therefore it seems likely that as many as 50 samples (about one a week) might be needed annually at each station. The frequency could later be reduced at many of the sites, possibly to once a month, and for some sites fewer than 50 samples might adequately define the system.

Although heavy metal studies have been emphasized in this discussion, the general sampling techniques and site selection are appropriate to other constituents of concern. Thus, as technology and financing may permit, a wide range of organic or inorganic components might be studied.

Table 3.1 Proposed sampling sites for baseline studies of river contributions to the marine environment; United States.

Station	Name and location	Drainage area Km^2	Mean discharge m^3/sec	Data Available			Priority
				Chem. qual.	Sed.	Minor elements	
51010	Penobscot R. at W. Enfield, Me.[2]	17,100	326	Yes	No	-	-
51012	Kennebec R. at Bingham, Me.[2]	7,040	121	Yes	No	-	-
51013	Androscoggin R. near Auburn, Me.[2]	8,460	168	Yes	No	[5]	[4]
56786	Merrimack R. at Lowell,Mass.[1]	12,000	201	Yes	Yes	-	-
63528	Connecticut R. at Thompsonville, Conn.[2]	25,000	445	Yes	No		
New	Hudson R. at Poughkeepsie, N.Y.[1]	21,000	360	No	No	[5]	[4]
51178	Delaware R. at Trenton, N.J.[1] [3]	17,600	327	Yes	[7]	[6]	[4]
54003	Schuylkill R. at Belmont Place, Phila., Pa.[2]	4,900	81	Yes	[7]	[6]	-
54004	Susquehanna R. at Harrisburg,Pa.[2]	62,400	962	Yes	Yes	[6]	[4]
55078	Potomac R. at Great Falls, Md.[1]	29,900	310	Yes	No	-	[4]
62936	James R. at Cartersville, Va.[2]	16,200	199	Yes	No		
52130	Roanoke R. at Scotland Neck,N.C.[2]	21,800	231	Yes	No	[5]	
52159	Tar R. at Tarbora, N.C.[2]	5,550	65	Yes	Yes		
52281	Cape Fear R. at Acme, N.C.[1]	8,920	96	Yes	No	[6]	[4]
54435	Pee Dee R. at Pee Dee, S.C.[2]	22,900	265	Yes	Yes	[6]	
67023	Savannah R. near Clyo, Ga.[2] [3]	25,500	336	Yes	No	[5]	[4]
51018	Altamaha R. at Doctortown,Ga.[2]	35,200	379	Yes	Yes	-	
53134	St. Johns R. at Jacksonville, Fla.[2]	22,000	170	Yes	No	-	[4]

Table 3.1 (continued)

Station	Name and location	Drainage area Km^2	Mean discharge m^3/sec	Data Available Chem. qual.	Sed.	Minor elements	Priority
53178	Miami Canal at NW 36th St., Miami, Fla. 2/	-	6.4	Yes	No	-	-
53204	Peace R. at Arcadia, Fla.2/	3,540	36	Yes	No	-	4/
53273	Suwannee R. at Bradford, Fla.2/	25,200	299	7/	No	-	4/
53276	Apalachicola R. at Chattahoochee, Fla.2/	44,500	457	Yes	7/	-	4/
55035	Escambia R. at Century, Fla.2/	9,880	173	Yes	No	-	-
50079	Alabama R. at Claiborne, Ala.1/	57,000	907	Yes	No	6/	4/
50128	Tombigbee R. near Jackson, Ala.2/	39,900	622	Yes	No	5/	4/
67946	Pascagoula R. at Hwy 26 near Benndale, Miss.1/	17,100	269	7/	No	-	
53935	Pearl R. naer Bogalusa, La.2/	17,200	251	Yes	Yes	-	
54880	Mississippi R. at Tarbert Landing, Miss.1/	3,220,000	14,000	Yes	Yes	-	4/
60143	Atchafalaya R. at Krotz Springs, La.2/ 3/	-	4,770	Yes	No	-	4/
52768	Sabine R. near Ruliff , Texas2/	24,200	239	Yes	No	6/	
52771	Neches R. at Evadale, Texas 2/	20,600	176	Yes	No	6/	
52781	Trinity R. at Romayor, Texas2/	44,500	202	Yes	Yes	6/	4/
56248	Brazos R. near Rosharon, Texas1/	114,400	207	Yes	No	6/	4/
52825	Colorado R. at Wharton, Texas2/	107,200	81	Yes	No	6/	-
54998	Rio Grande R. at Brownsville, Texas2/		19	Yes	Yes	-	4/

Table 3.1 (continued

Station	Name and location	Drainage area Km^2	Mean discharge m^3/sec	Data Available Chem. qual.	Sed.	Minor elements	Priority
68019	Colorado R. at Southern International Boundary, Arizona[1]	629,000	10	Yes	No	5	-
60293	Los Angeles R. at Pacific Coast Hwy, Long Beach, Calif.[1]	2,150	3.2	Yes	No	5	4
51508	San Joaquin R. near Vernalis, Calif.[1]	35,100	124	Yes	Yes	6	4
51605	Sacramento R. at Sacramento, Calif.[1]	61,000	647	7	Yes	5	4
51658	Eel R. at Scotia, Calif.[2]	8,070	200	Yes	Yes	6	
51679	Klamath R. near Klamath, Ca.[2] [3]	31,400	488	Yes	7	6	4
51906	Chehalis R. at Porter, Wash.[2]	3,350	120	Yes	Yes	6	
51963	Skagit R. near Mount Vernon, Wash.[2]	8,010	468	Yes	No	6	4
New	Columbia R. at the Dalles, Oregon[1] [3]	614,000	5,530	7	No	5	4
New	Wilamette R. at Wilsonville, Oregon[2]	21,800	833	No	Yes	6	4
54239	Umpqua R. near Elkton, Oregon	9,530	212	Yes	Yes		
54238	Rogue River near Agnes, Oregon	10,200	179	Yes	No		
54052	Yukon R. at Ruby, Alaska	517,000	3,670	Yes	Yes		4

Table 3.1 (continued)

Station	Name and location	Drainage area Km^2	Mean discharge m^3/sec	Data Available Chem. qual.	Sed.	Minor elements	Priority
54055	Kuskokwim R. at Crooked Creek, Alaska	80,500	1,274	Yes	Yes		
63414	Copper R. near Chitina, Alaska	53,400	1,056	Yes	Yes		
63425	Koyokuk R. at Hughes, Alaska	48,400	532	No	Yes		4/

1/ Proposed for 1973 NSQA network (or is equivalent to an NSQA site).
2/ Proposed for later addition to NSQA network.
3/ IHD station.
4/ Suggested for top priority in IDOE network.
5/ Some heavy metal data available for upstream station.
6/ " " " " " " this sampling point.
7/ Some data available in past but not in current records.

Sewer Outfalls

The following types of outfalls can exist in coastal areas: (1) untreated sanitary outfalls, (2) untreated industrial outfalls, (3) municipal sanitary and industrial sewer outfalls from treatment plants exclusive of storm runoff outfalls (4) storm sewer outfalls, (5) combined municipal, sanitary, industrial and storm sewer outfalls.

(1) It is virtually impossible to sample untreated sanitary outfalls when they are present in large numbers along a coast. Where they are localized, however, they should be sampled on a time scale discussed below.

(2) Untreated industrial outfalls are either emptied continuously into the coastal water or programmed to coincide with a natural cycle such as ebb tide. For continuous or programmed flushing, a knowledge of the expected concentration of the major pollutant gained either from assay of the outfall or from information provided by the factory management should be used. Normally, once determined it can be used in assessing the burden to the estuary on the basis of production schedules.

(3) Municipal and industrial sewer outfalls from treatment plants should be sampled systematically as described below to develop a sense of the ambient flux of materials to coastal waters with changing relative contribution from the two sources.

(4) Storm sewers should be analyzed if possible after each major storm and on a more systematic basis during the spring in the northern states. Volumes of flow should be sought at the same time that chemical data are obtained.

(5) Combined, treated sewer outfalls should be measured systematically.

The systematic sampling should probably be done on a weekly basis. Treatment plant data may be obtained from the local commissioner of sewers or the resident engineer who can also provide the requisite aliquots of material to be analyzed.

It is estimated that an estuary like Long Island Sound, with its surrounding high population density, has about fifteen major municipal sewer outfalls discharging directly into it. All of these should be sampled to get an idea of the burden of pollutant transmitted to the Sound in addition to the flow from adjacent water bodies and streams.

Perhaps a total of 100 major sewer outfalls discharge into coastal waters in the United States. They should all be assayed for the critical substances and flux rates.

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4

ANALYTICAL TECHNIQUES FOR SELECTED INORGANIC SPECIES

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Introduction

This chapter describes the measurement of trace metal constituents in the marine environment by neutron activation analysis and by spectrophotometric techniques. Emphasis has been placed on the measurement of those elements of present environmental concern.

The procedures are described with the assumption that samples have been carefully collected and properly preserved (see Chapter 6). We emphasize that analyses of samples contaminated during collection and handling can only waste time and effort and lead to misleading conclusions. We intend that these procedures be useful to analytical chemists already familiar with the instrumentation and general principles involved.

Neutron Activation Analysis (David E. Robertson and Roy Carpenter)

Anthropogenic wastes enter the marine environment from river drainage, from the atmosphere, and from direct coastal discharges. Once these pollutants have entered the oceans, they become entrained in complex biogeochemical and physical processes which serve to either disperse or reconcentrate the various contaminants. An essential feature of an effective marine environmental quality monitoring program is the capability for measuring these ocean pollutants at near-background

levels in the main matrices involved in marine pollution--fresh waters, sea water, atmospheric particulates, biological organisms, and sediments.

In monitoring the marine environment for metallic pollutants, few analytical methods offer the versatility and sensitivity achieved by neutron activation analysis (NAA). With the recent technological advances in developing high resolution solid state Ge(Li) detectors and their associated electronics, it is now possible to measure a wide spectrum of elemental constituents in virtually any environmental media by nondestructive, instrumental neutron activation analysis (INAA). Multi-element analyses by this method are advantageous in two respects: (1) many of the potentially hazardous pollutant trace metals in various environmental matrices can be simultaneously measured and (2) many of the innocuous elements which greatly affect the biological availability and the biogeochemistry of the pollutant metals can likewise simultaneously be measured. When the concentrations of metallic pollutants are below the detection limits afforded by nondestructive INAA, either preconcentration or postirradiation separations of the elements of interest can be performed. In most cases, this increases the sensitivity by orders of magnitude.

This treatise describes the technology and applications of NAA for the measurement of trace metal constitutents in the marine environment. The contents are organized to indicate which trace elements can normally be measured by nondestructive INAA in the five matrices considered, and, when necessary, the chemistry required for preconcentrations and separations is provided. Emphasis has been placed on the measurement of the most potentially hazardous trace metals Hg, Cd, Cu, Ag, As, Sb, Cr, Se and Zn. Lead and Sn are not included since NAA is not a sensitive method for these elements.

Principles of Neutron Activation Analysis

Neutron activation analysis is a nuclear method of elemental analysis in which the elements in a sample to be analyzed are made radioactive by irradiation with neutrons, and the induced radioactive species are then identified and measured. The amount of a given neutron activation product that is formed during neutron irradiation is directly proportional to the amount of its parent isotope. Measurement of the radionuclide provides a measure of the total concentration of the parent element.

The basic equation for activation is

$$W = \frac{AM}{\sigma f \phi (1-e^{-\lambda t}) x 6.02 x 10^{23}}$$

where W = weight of element irradiated, in grams

A = induced activity in disintegrations/sec at the end of irradiation

f = flux of neutrons used in the irradiation, in neutrons/cm^2/sec

σ = the activation cross section for the nuclear reaction concerned, in cm^2

ϕ = the fractional abundance of the particular isotope of the element concerned

M = atomic weight of that element

λ = the decay constant of the induced radionuclide, in sec^{-1}

t = irradiation time, in seconds

From the above equation it is obvious that the detection sensitivity for a given element can be improved by increasing the neutron flux and/or the irradiation time. The relative detection sensitivities for all of the elements in the periodic table are a function of their activation cross section for the nuclear reaction concerned, the decay constant of the induced radionuclide, the atomic weight of that element and the fractional abundance of the particular isotope of the element

concerned. For most elements, NAA is an extremely sensitive method of analysis, as evidenced in Tables 4.1-7. A notable exception is lead, an important ocean pollutant, which is best measured by isotope dilution-mass spectrometry or possibly atomic absorption spectrometry.

The concentration of an element in a sample can be calculated from the above equation by using a flux monitor and if the efficiency of the counter for the particular activation product is known. However, the easiest and most accurate method for quantifying the concentration of any element is to include in the same irradiation of the sample a standard of known weight of the element being determined. Then the weight of the element being determined is obtained as follows:

$$\frac{\text{Weight of element in unknown}}{\text{Weight of element in standard}} = \frac{\text{Activity of element in unknown}}{\text{Activity of element in standard}}$$

This method obviates the need for careful neutron flux monitoring and counting instrument calibrations, since the only measurements that are required are the relative counting rates of the neutron activation products of the element of interest in the unknown and in the standard. This comparative method requires that the sample and standards be both irradiated and counted under as nearly as possible the same conditions.

The neutron activation method of analysis does not distinguish between various physicochemical forms of an element in a particular sample, but provides a measure of its "total" concentration. However, if different physicochemical specimens of the same element are separated prior to the neutron irradiation, NAA can provide a measure of each specific form.

In practice, the fundamental steps in a typical NAA are:

(1) Irradiate weighed quantities of the sample and standard in suitable containers for a sufficient time to give adequate radioactivity for the element to be determined.

(2) After the irradiation, INAA is accomplished by simply counting the samples and standards on

Ge(Li) and/or NaI(Tl) gamma-ray spectrometers at optimum times following the irradiation to directly measure elements producing short, intermediate and long-lived neutron activation products.

(3) Compare the radioactivity of samples and standards under identical counting conditions, making decay corrections, Compton corrections and background corrections when necessary.

(4) If INAA does not provide the desired sensitivity radiochemically separate the activation product(s) of interest from the interferring radionuclides and count the separates in the same geometries as the standards.

(5) To ensure that the gamma-ray being measured is due solely to the radionuclide of interest, check the half-life of the activity to be certain it is decaying at the proper rate. Further confirmation may be desired when the radionuclide emits more than one gamma ray per disintegration, in which case both the energies and relative intensities can be used for verification.

For more detailed discussions of the basic theory, fundamentals and applications of NAA, the readers are referred to the following references: Anonymous (1971); Barbier (1969); DeVoe and LaFleur (1969); Guinn and Lukens (1965); Kruger (1971); Lenihan and Thompson (1969); Lutz *et al.* (1971); Rakevic (1970); Ryan (1973) and Smales (1967).

Neutron Irradiation Facilities and Procedures

The most generally applicable neutron source for trace level activation analysis is the high flux nuclear reactor. Most of these reactors normally operate at power levels in the range of 10 to 1000 kilowatts and produce thermal neutron fluxes of 10^{11} to 10^{13} n/cm^2/sec. Reactors used in such work are generally of the research type and are becoming increasingly available for use by the scientific community. The neutron flux in such reactors does vary spatially about the reactor core and inhomogeneities in the flux must be

taken into account. Such inhomogeneities can be minimized by rotating the samples in the flux field in various types of specimen racks designed for that purpose. In a typical irradiation, up to 48 samples and standards can be irradiated in one rotator simultaneously. Although the horizontal neutron flux gradients in each rotator are minimized by revolving the samples, there exists a small vertical flux gradient which must be accounted for by placing a standard at each tier of samples if they are stacked vertically.

"Rabbit" irradiations are used for activating samples for periods of seconds to minutes, where the induced activity of interest is a short-lived isotope (one having a half-life in the range of seconds to minutes). Samples are activated and counted one at a time (usually purely instrumentally) with the reactor operating steadily. A standard sample of the element of interest is similarly activated and counted at intervals.

Sample Preparation

A. Materials and Manipulations

A tremendous advantage of using INAA is that sample handling and manipulations are reduced to a minimum, thereby greatly reducing the probability of contaminating a sample. Contamination problems are particularly severe when dealing with natural waters. The problems of contamination in trace element analysis have been dealt with in the literature (Robertson, 1968; 1972; Thiers, 1957 a,b). Solid samples, such as sediments, biological tissue, air filters and sea and fresh water salts are normally weighed and heat sealed in cleaned polyethylene, polypropylene, or high purity quartz vials ranging in size from 1 cc to 10 cc. Sample size can range from a few milligrams to tens of grams depending upon the sensitivity required and sample size available. Desiccation of sediments and biological tissue prior to encapsulation is accomplished by either freeze-drying or oven-drying at 105°C. Special precautions are

necessary when drying samples for trace Hg analyses to avoid losses by volatilization. These precautions are discussed in the section dealing with mercury analysis by NAA.

Natural waters, especially seawater, must usually be evaporated to dryness if the samples are to be irradiated for an extended period, since gaseous radiolysis products (H_2, O_2 and other gases) are generated and produce high pressures which rupture the irradiation vials. Desiccation is accomplished by either freeze drying or slowly evaporating the water under an infra-red heat lamp. Again, great care must be taken not to volatilize Hg and other volatile elements during these drying operations.

Water samples can be neutron irradiated directly, without evaporation, if the water is sealed in quartz vials. However, in the case of seawater, the radiolysis products build up dangerously high pressures which have even shattered sealed quartz vials, and this method is not recommended for routine use. In addition, quartz contains much higher levels of impurities than polyethylene or polypropylene and is a potential source of contamination.

Tanner *et al.* (1972), and Brune and Landstrom (1966) have described a method for neutron irradiating 30 to 80 ml samples of fresh water in a frozen state in polyethylene containers as a means of preserving the samples prior to and during the neutron irradiation.

It is important that samples in a given set be encapsulated in reproducible, identical geometries so that all samples will be neutron irradiated in a known orientation. Elemental standards should be encapsulated and irradiated as identically as possible to the samples. Standards should be prepared in a matrix as close to the sample matrix as practically possible in order to minimize problems such as neutron self-shielding, irreproducible

activation geometries and interferring nuclear reactions. For example, when neutron activating large quantities of sea salts, the elemental standards should be added to an equivalent amount of NaCl encapsuled in the same geometry as the sample. However, if sample sizes are kept very small, matrix matching is not a critical requirement for the types of samples normally encountered in the marine environment.

The use of standard reference materials of known elemental composition as activation standards is a convenient method of simulating the sample matrix. A wide variety of reference materials is available for use as biological and geological standards. The National Bureau of Standards supplies orchard leaf, tuna meal and bovine liver as standard reference materials for analysis of biological material. The elemental concentrations in these materials are gradually being certified by the National Bureau of Standards and may become useful activation standards in the future. The U. S. Geological Survey supplies a wide variety of geological standard reference materials which have reasonably well documented elemental concentrations (Flanagan, 1969; Fleischer, 1965). These rock samples are non-uniform for some elements, but represent the best available reference materials for analyses of marine sediments and atmospheric particulates. The Environmental Protection Agency (EPA) has provided three freshwater sediments which many laboratories have analyzed for mercury. Two have mercury levels of ∿45 and ∿105 ppm Hg, respectively, which are much higher concentrations than normally found in natural sediments. The third has ∿0.06 ppm and is at the lower end of most mercury values in sediments. These sediments are available in limited amounts from J. H. Finger, Environmental Protection Agency, Surveillance and Analyses Division, Athens, Georgia. Frequently, however, individual elemental standards must be prepared and included with the irradiations

when the concentrations of those particular elements are not sufficiently high in the standard reference materials to be accurately measured.

To date, no standard reference natural water samples are available, and analysts are required to prepare their own elemental standards. However, the EPA has made available six water reference samples which, when diluted as prescribed, give different concentrations of As, Cd, Cr, Cu, Pb, Se and Zn in the ppb concentration range. These samples are available from J. A. Winter, Methods Performance Evaluation Activity, National Environmental Research Center, EPA, Cincinnati, Ohio (45268).

B. Preconcentration and Postirradiation Separations

In most cases the only matrices that require preconcentration of some trace elements prior to neutron activation are fresh and sea waters, where the natural elemental concentrations are near or below the parts per billion range, thus requiring large volumes of sample to achieve the desired sensitivity. Atmospheric particulates are naturally preconcentrated from large volumes of air during their collection on various filter media. Generally, the trace metal levels in marine organisms, sediments and atmospheric particulates are sufficiently high that convenient sized samples can be neutron activated and postirradiation radiochemical separations be performed.

Postirradiation separations are usually the most desirable, because once the radioactive species are produced, any amounts or types of stable reagents and labware can be used in the chemical manipulations without contaminating the samples with the elements being measured. However, in the case of sea salts, if trace elements with short-lived (<1 to 2 days) neutron activation products are to be measured, the trace elements must be separated from the NaCl matrix before neutron irradiation, since high radiation doses result from the large concentrations of

^{24}Na and ^{38}Cl which are produced when sea salts are neutron activated.

Preconcentration schemes for natural waters can be very simple, involving perhaps only the removal of much of the major activity component, such as ^{24}Na. Alternatively, the separations can be made groupwise, and both such procedures are usually followed by gamma-ray spectrometry to obtain final specific measurement of each individual neutron activation product. Occasionally, and especially where the highest possible sensitivity is required, very thorough preconcentration or postirradiation separations are necessary. In the following sections chemical separation schemes are given for those elements of interest which cannot be measured in the various matrices purely by INAA.

Gamma-Ray Spectrometry and Data Handling

Since all of the elements of interest in this work, except Pb and Sn, form neutron activation products with easily measurable gamma-rays, this section will be concerned only with gamma-ray spectrometry involving solid state Ge(Li) diode detectors and NaI(Tl) scintillation crystals.

Lithium drifted germanium detectors have revolutionized INAA because of their excellent energy resolving capability (about 2 to 3 kev FWHM above about 200 kev). Even better resolutions can be attained at energies below about 200 kev by utilizing thin germanium low energy photon detectors (LEP detectors). These detectors will allow increasing use of low energy gamma-rays and x-rays for INAA. Heretofore, single NaI(Tl) crystals were capable of resolving only a few of the major constituents in complex mixtures of radionuclides. Ge(Li) detectors, however, can resolve the gamma rays of nearly all neutron activation products formed in marine environmental matrices, if their concentrations are sufficiently high to be detected. A good example of this improved resolution is shown in Figure 4.7, which compares the gamma-ray spectra of neutron activated sea salts counted on both a NaI(Tl) crystal and a

Ge(Li) diode detector. The main disadvantage of Ge(Li) detectors is their relatively low efficiency, compared to NaI(Tl) crystals. However, for most neutron activated samples the lower efficiency of the Ge(Li) detectors is not a serious problem, since sufficiently high concentrations of the neutron activation products of the elements of interest can normally be produced in high flux nuclear reactors.

The basic principles and applications of Ge(Li) diode detectors have been adequately described in the literature (Bertolini and Coche, 1968; Brown *et al.*, 1969; Camp, 1967; Cooper, 1973; Dearnaley and Northrop, 1966; Heath, R. L., 1969; Hollander, 1966; Keil and Berndt, 1972; Lutz *et al.*, 1971). Briefly, the hardware required for a basic Ge(Li) gamma-ray spectrometer is the Ge(Li) detector, a preamplifier, an amplifier, and a 2000 or 4000 channel pulse height analyzer.

While Ge(Li) detector systems of greater versatility and sophistication have been constructed (for example, Cooper and Perkins, 1972) ordinary Ge(Li) detector systems are adequate for measuring a large group of neutron activation products in environmental samples.

In practice, the neutron activated samples and standards are transferred from the irradiation containers into standard counting geometries, which may consist of polyethylene vials of various sizes or some other suitable containers. Care must be taken to assure that the samples and the standards are encapsulated in identical counting geometries. Relatively small differences in orientation of the samples and standards within the containers can lead to serious errors if the containers are counted on or close to the Ge(Li) detector. This is especially important for such unusually shaped samples such as filters and resins. One must not be seduced into placing the samples on the detector to increase counting efficiency without experimentally evaluating the geometry factors. The samples and standards are then positioned on or near the Ge(Li) detector in a fixed geometry and counted for a sufficient length

of time to achieve the necessary counting statistics. Differences in geometries in a set of samples can be minimized by counting the samples several cm away from the Ge(Li) detector if sufficient activity is present.

NaI(Tl) detector systems are most useful when senstivity is the main concern, since NaI(Tl) detectors are approximately 10 to 100 times more efficient than Ge(Li) diode detectors. NaI(Tl) crystals of various geometries are commercially available, but the most commonly employed are well crystals or solid, cylindrical crystals of various sizes normally ranging from about 3 to 13 inches in diameter and about 3 to 9 inches thick. The background and Compton response of these crystals can be substantially lowered by anticoincidence shielding with large plastic phosphors or annular NaI(Tl) crystals (Perkins and Robertson, 1965; Wogman *et al.*, 1967, for examples). Two large anticoincidence-shielded NaI(Tl) detectors can be designed to count as dual coincidence gamma-ray spectrometers, thereby greatly increasing the sensitivity and selectivity of NaI(Tl) gamma-ray spectrometry (Perkins and Robertson, 1965).

These large crystal NaI(Tl) systems are particularly applicable for measuring traces of neutron activation products which have been radiochemically separated from an interferring matrix. Their high efficiencies permit very low levels of radioactivity to be measured in relatively short counting intervals.

Interpretation of gamma-ray spectra obtained from NaI(Tl) and Ge(Li) detectors typically involves location of peaks in the spectra, determination of peak energies, measurement of peak areas, and calculation of concentrations of the elements from which the gamma-ray emitters were formed. In NaI(Tl) spectrometry, if the radionuclide mixture is quite simple, the peak areas can be obtained by manual subtraction of background and Compton contributions. However, if the radionuclide mixture contains more than 3 or 4 gamma emitting radionuclides, the spectrum stripping is most conveniently and accurately accomplished by using a computer program designed for that purpose. Because

peak areas in Ge(Li) diode spectra are normally well resolved, determination of peak intensities by manual subtraction of the background and Compton contribution can be readily accomplished in even very complex mixtures of radionuclides. Normally, an equal number of channels on each side of the peak area are selected to represent the contribution due to background and Compton interferences and this contribution is manually subtracted from the peak area. However, if many routine analyses are required, the manual method can become tedious and time consuming and the most rapid and economical way to process the data is with a computer.

The development and application of computer programs for the analysis of gamma-ray spectra are, by no means, elementary tasks. For computer analysis to be workable, spectrometer systems must be very electronically stable, precisely adjusted before counting, and the spectra must be converted to a computer compatible form. Incorrect input of data confuses a computer, resulting in termination of the analysis or computation of wrong answers. Once these problems have been overcome, a computer analysis of the gamma-ray spectra is capable of rapidly converting the spectra directly into concentrations of individual constituents. However, unless one is tooling up for the analysis of a large number of routine samples, it is often more trouble than it is worth to develop a computer program designed to quantify INAA.. It is a good practice to check computer programs by hand calculations occasionally to ensure that they are operating as designed.

For more detailed discussions of the use of computers for data reduction the readers are referred to the following material: DeVoe and LaFleur, 1969; Salmon and Creevy, 1970; O'Kelley, 1962.

Elemental Analyses

Fresh Waters

A. Instrumental Neutron Activation Analysis

Nearly all of the potential pollutant elements of interest can be measured in fresh waters (atmospheric precipitation, rivers, lakes, tap water, etc) by INAA utilizing a very

simple radiochemical group separation to remove interferring neutron activation products. Figure 4.1 illustrates the relative abundances of the major and some of the minor radionuclides formed during neutron activation of Columbia River water and their change in concentration with time. It is obvious that the high activity due to ^{24}Na will mask the gamma-ray spectra of all of the trace neutron activation products of the elements of interest, except ^{38}Cl, ^{42}K, ^{56}Mn and possibly ^{87m}Sr. Therefore, to measure the short-lived neutron activation products of As, Hg, Cu, Zn, Ba and La, as well as several other elements of geochemical interest, it is necessary to remove the interferring ^{24}Na.

Tanner *et al.* (1972), have described a simple two-step separation scheme which eventually permits the semi-instrumental measurement of about 19 trace elements (see Figure 4.2). The method simply consists of passing a neutron activated water sample, containing carriers of 0.1 mg each of Na, K, Rb and Cs through a column of Dowex 1x2 anion exchange resin which has been converted to OH^-, $S^=$ and $CO_3^=$ forms by contacting with 1M solutions of NaOH, Na_2S, Na_2CO_3, respectively. All trace activation products, except the alkali metals and the alkaline earths, are retained on the resins by precipitation-ion exchange mechanisms. The combined resin is then rinsed with distilled water, transferred into a plastic counting container, and counted directly on a Ge(Li) detector. The column effluent, containing the radionuclides ^{24}Na, ^{42}K, ^{86}Rb, ^{134}Cs and traces of neutron activation products of the alkaline earths Ca, Ba and Sr, is treated with an excess of 4% sodium tetraphenylboron to precipitate Cs, K and Rb. The precipitate is washed, filtered, and counted directly on a Ge(Li) detector. Standard solutions of the elements of interest are sealed in quartz vials, irradiated, transferred to plastic counting containers, and counted in

Figure 4.1 RELATIVE CONCENTRATIONS OF INDUCED RADIONUCLIDES IN RIVER WATER FOLLOWING AN INTEGRAL THERMAL NEUTRON IRRADIATION OF 2 x 10^{16} n/cm^2. EMPHASIZING THE PROBLEM OF MEASURING SHORT-LIVED ACTIVATION PRODUCTS BY INAA IN THE PRESENCE OF HIGH CONCENTRATIONS OF SODIUM-24

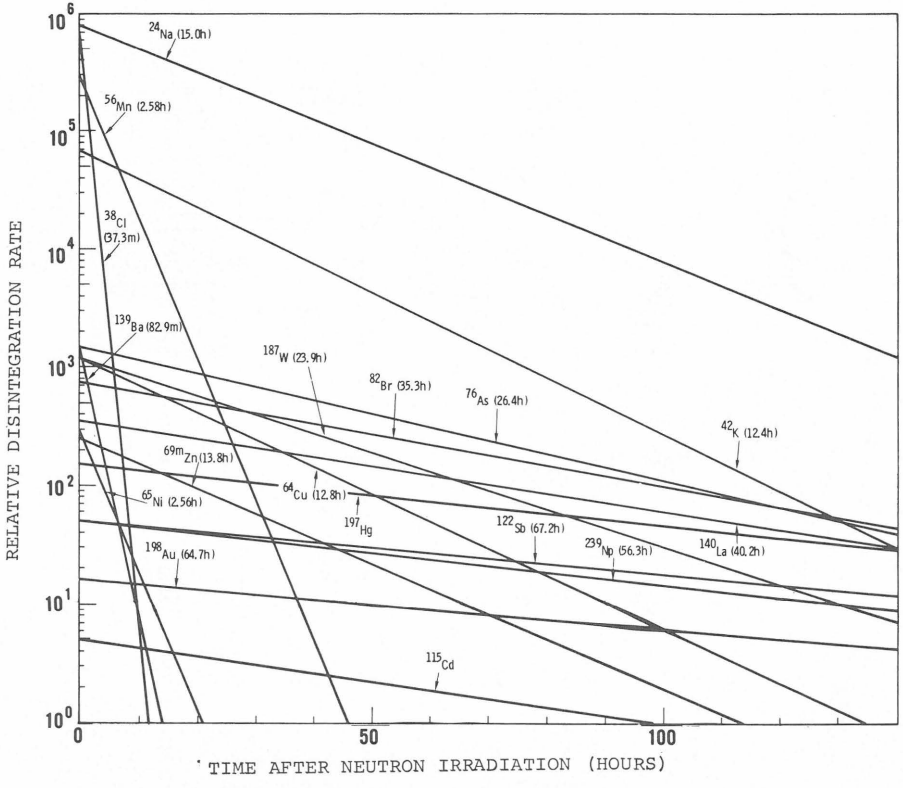
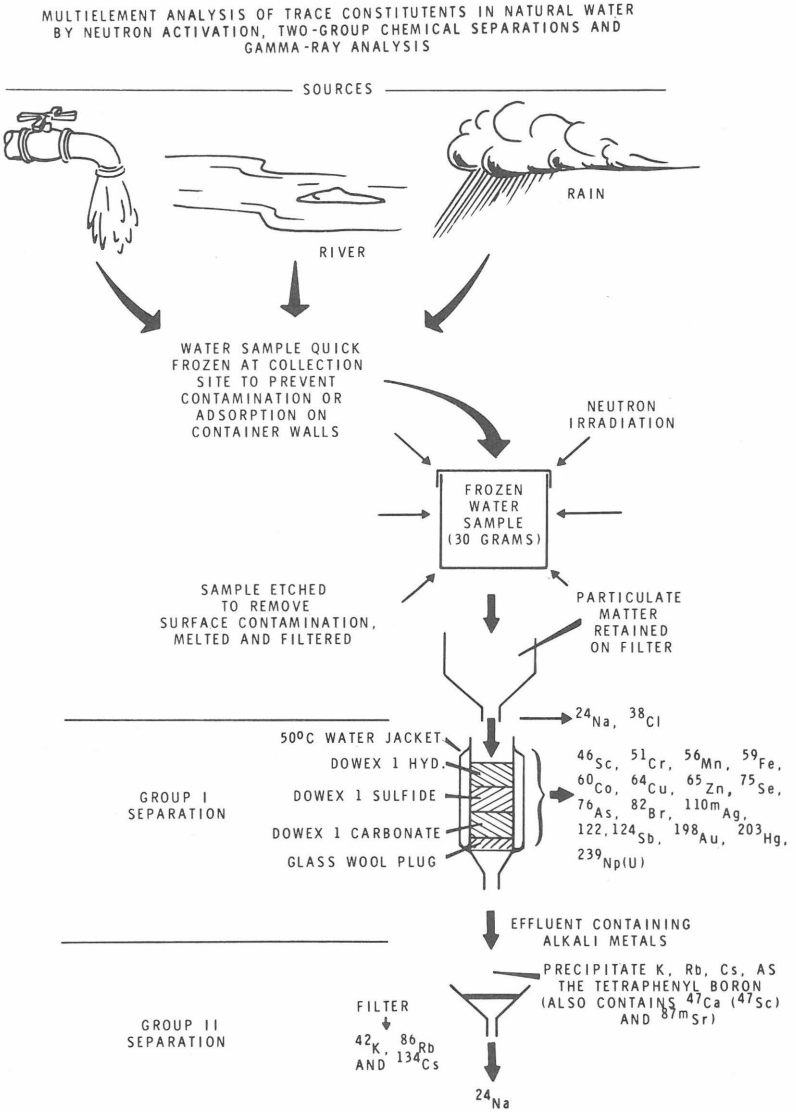


Figure 4.2


MULTIELEMENT ANALYSIS OF TRACE CONSTITUTENTS IN NATURAL WATER BY NEUTRON ACTIVATION, TWO-GROUP CHEMICAL SEPARATIONS AND GAMMA-RAY ANALYSIS
SOURCES
RAIN
RIVER
WATER SAMPLE QUICK FROZEN AT COLLECTION SITE TO PREVENT CONTAMINATION OR ADSORPTION ON CONTAINER WALLS
NEUTRON IRRADIATION
FROZEN WATER SAMPLE (30 GRAMS)
SAMPLE ETCHED TO REMOVE SURFACE CONTAMINATION, MELTED AND FILTERED
PARTICULATE MATTER RETAINED ON FILTER
^{24}Na, ^{38}Cl
50°C WATER JACKET
DOWEX 1 HYD.
DOWEX 1 SULFIDE
DOWEX 1 CARBONATE
GLASS WOOL PLUG
GROUP I SEPARATION
^{46}Sc, ^{51}Cr, ^{56}Mn, ^{59}Fe, ^{60}Co, ^{64}Cu, ^{65}Zn, ^{75}Se, ^{76}As, ^{82}Br, ^{110m}Ag, $^{122,124}Sb$, ^{198}Au, ^{203}Hg, ^{239}Np(U)
EFFLUENT CONTAINING ALKALI METALS
PRECIPITATE K, Rb, Cs, AS THE TETRAPHENYL BORON (ALSO CONTAINS ^{47}Ca (^{47}Sc) AND ^{87m}Sr)
FILTER
^{42}K, ^{86}Rb AND ^{134}Cs
GROUP II SEPARATION
^{24}Na

the same geometries as the samples to quantify the measurements by the comparator method. Figure 4.3 illustrates the gamma-ray spectra of the short-lived radionuclides measured in neutron activated Columbia River water before and after the separation scheme. Figure 4.4 illustrates the gamma-ray spectra of the long-lived neutron activation products contained on the resins and tetraphenylboron precipitate by counting 23 and 24 days after the irradiation. Eleven additional neutron activation products can be detected from these counts.

In Table 4.1 the elements which can normally be measured in fresh waters by this semi-INAA technique are indicated, together with the detection limits of many of the elements of interest. Without any separations at all it would still be possible to measure Ag, Br, Cl, Co, Cr, Cs, Fe, Mn, Na, Rb, Sb, Sc, Se, U, Zn, and occasionally Hg, simply by counting the neutron activated river water samples at several optimum times following the irradiation (approximately 4 hours, 3 days and 20 days). The radiochemical separations permit the measurement of As, Cu, La and K.

Table 4.1 also lists the approximate limits of detection for 20 elements in river water utilizing the method of Tanner *et al.* (1972) and compares these limits with maximum allowable concentrations of some of the more toxic heavy metals in drinking water, as established by the U. S. Public Health Service. As indicated in Table 4.1, this semi-INAA technique can be used to detect most heavy metal pollutants in fresh waters at concentrations well below maximum permissible limits. Of the most toxic heavy metals, only Cd, Pb, Sn and occasionally Hg cannot be measured by this method.

Edgington and Lucas (1971) and Girardi and Sabbioni (1968) have developed a method of removing ^{24}Na, the

Figure 4.3 **GAMMA-RAY SPECTRUM OF NEUTRON ACTIVATED RIVER WATER**

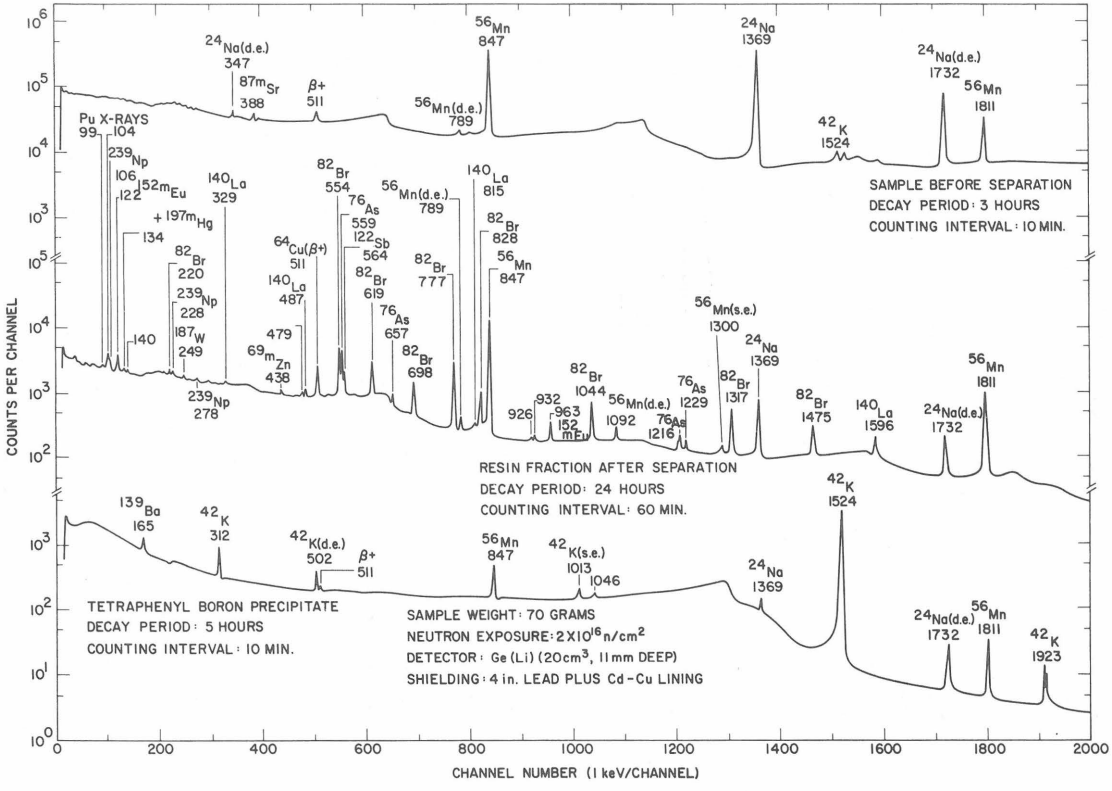


Figure 4.4 **GAMMA-RAY SPECTRUM OF NEUTRON ACTIVATED RIVER WATER**

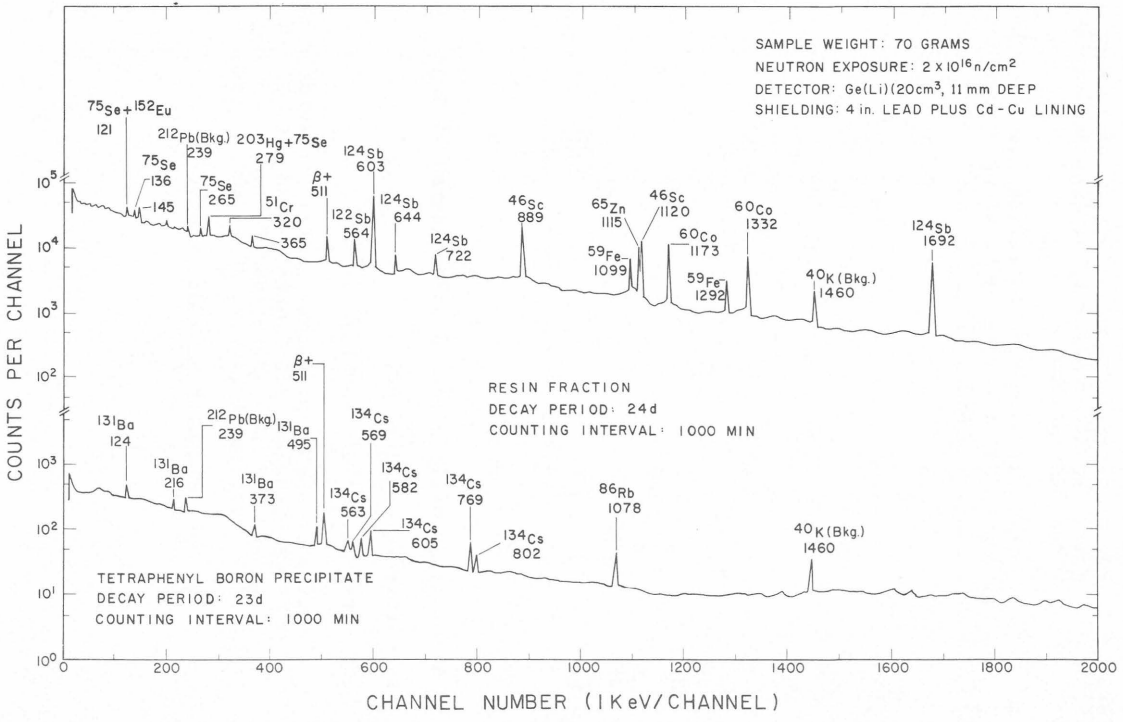


Table 4.1 Approximate Limits of Detection of Various Trace Metals in River Water by Neutron Activation and Group Separations

Element	Approx. Estimate of Conc. in River Waters (µg/l) (a)	Detection Limits by NAA After Group Separation (µg/l) (b)	Drinking Water Allowable Limits (µg/l) (c)
Ag*	0.3	0.01	50
As	2	0.1	50
Ba	10	5	1000
Br*	20	0.01	
Cd	--	50	10
Cl*	7800	100	
Co*	0.2	0.01	
Cr*	1	0.1	50 (Cr^{+6}
Cs*	0.02	0.01	
Cu	7	0.1	1000
Fe*	--	10	300
Hg	0.07	0.1	(10)
K*	2300	10	
Mn*	7	0.01	50
Na*	6300	10	
Rb*	1	0.1	
Sb*	0.3	0.01	
Sc*	0.004	0.001	
Se*	0.2	0.1	10
Zn*	20	1	5000

* Elements normally detected and measured by INAA

(a) after Turekian (1969)

(b) after method of Tanner *et al.* (1972)

Thirty to eighty ml water samples; integrated thermal neutron exposure 2×10^{16} n/cm^2; count for 10 min., 60 min., and 1000 min. after decay times of 3 hrs, 24 hrs and 23 days, respectively; 20 cc Ge(Li) detector.

(c) U. S. Public Health Service (1962).

major interference in NAA of environmental samples, based on selective retention of the ^{24}Na on hydrated antimony pentoxide (HAP). All neutron activation products, except ^{24}Na, ^{182}Ta and ^{233}Pa, reportedly pass through the HAP column and are measured by Ge(Li) gamma-ray spectrometry. The method of Edgington and Lucas (1971) provides a breakdown into further group separations by selective elution of the effluent from the HAP separation from Dowex 1x8 anion exchange resin.

Neutron activation analysis is not a sensitive method for Pb and Sn, but Cd can be measured by preconcnetration from 500-1000 ml of fresh water. Since Hg and Ag are normally present in uncontaminated fresh waters at concentrations near or below their detection limits for INAA, preconcentration or postirradiation separations are described below for these elements, as well as for Cd.

Suspended Particulate Matter

Particulate matter suspended in natural waters carry significant percentages of the total amounts of several elements in the waters. To determine the partitioning of the elements between the two phases, it is desirable to isolate the suspended particulate material, by either centrifugation or membrane filtration. The preferred method is by membrane filtration, and the usual filter pore size used is about 0.3 to 0.5 μ. Both Millipore® and Nuclepore® filters have commonly been used for this purpose, and both filters contain roughly comparable traces of most impurities. It is recommended that as large a water sample as practically possible be filtered to obtain enough suspended matter for elemental analysis and to reduce the probability of contamination during the filtering process. The first several hundred ml of filtrate should be discarded because of contamination which may be leached from the filters.

Several types of all plastic filtering devices are commercially available (Millipore Corporation; General Electric Co., for examples) which are ideal for filtering natural waters. The usual procedure for INAA of suspended particulates is to collect the material on tared filters (Millipore,® Nuclepore,® etc.), desiccate to a constant weight and reweigh the filters to obtain a gravimetric analysis. Nuclepore® filters offer the most desirable taring characteristics because they absorb less moisture than other types of membrane filters. Weighing of membrane filters on an analytical balance may present some problems due to the electrostatic charge which accumulates on the filters. This can be overcome by inserting a special α-emitting Po source (Watson Brothers, Northern California Division, Scientific Instruments, San Mateo, California) inside the balance case. The ionization of the air produced by the α particles neutralizes the electrostatic charge on the membrane filter.

The weighed filters are then encapsuled in plastic irradiation vials and neutron activated, together with appropriate standards of the elements of interest. After the irradiation, the samples are then counted on a Ge(Li) detector at optimum decay times to measure up to 24 trace elements. Since the suspended particulate matter in natural waters is usually a combination of both geological and biological material, the discussion of which elements can be measured and the detection sensitivities which can be achieved will be covered in the following sections concerned with analyses of biological and geological material.

B. Analysis of Elements Requiring Preconcentration or Post-irradiation Separations

The trace metals of pollution concern which frequently need to be preconcentrated or separated from natural waters to achieve the necessary sensitivity for NAA are

Hg, Cd, Ag, As and Se. Procedures are given below which accomplish these separations.

Under certain conditions, mercury losses by diffusion through polyethylene irradiation containers during neutron activation of fresh waters have been reported (Bate, 1971). Individual investigators should evaluate their particular methods of encapsulating and irradiating natural waters to ensure that such losses are not significant. One method of minimizing such losses is to irradiate the water sample in a frozen state, as indicated in the following procedure.

Mercury Procedure I

Landstrom and Wenner (1965) have reported a simple procedure for the isolation of ^{203}Hg from neutron activated natural water samples. A 30 ml water sample, neutron irradiated in a frozen state for 12 hours at a flux of about 2 x 10^{12} n/cm^2/sec, is transferred to a distillation flask and acidified with H_2SO_4. Hydrogen peroxide and HBr are added and the Hg is distilled as the bromide. The distillate is made 3.5 M in H_2SO_4 and 0.1 M in HCl, H_2O_2 is added, Br is driven off, and the solution is passed through a small column of Dowex 2x10 anion exchange resin in the sulfate form. The column, containing the sorbed Hg, is rinsed with 0.1 N HCl to elute Sb, As and Se, and the anion resin is then counted to determine $^{197-203}Hg$ activity. Mercury standards, irradiated with the samples, are counted in the same geometry to quantify the measurements. Recovery is reported to be 96%.

Mercury Procedure II

Ljunggren *et al.* (1971), have reported a method for separating radiomercury from neutron activated fresh waters. Water samples 0.3 to 10 ml in volume are sealed in cleaned quartz vials and irradiated for 2 to 3 days in a thermal neutron flux of about 3-4 x 10^{12} n/cm^2/sec. After a short cooling period, the samples and 20 mg of

Hg carrier are digested in a Bethge apparatus with 15 ml of 10:1 mixture of concentrated nitric acid and sulfuric acid. To the digested solution is added a mixture of 5 ml of 70% perchloric acid and 0.5 gm of glycine in 5 ml of water. The Hg is distilled from the solution until the temperature reaches 250°C. The distillate (continaing the Hg) is diluted to about 300 ml with distilled water and Hg is electrodeposited on a tared thin gold foil. A platinum foil (2 x 3 cm) is used as an anode and a thin gold foil (1 x 3 cm) as the cathode, and these electrodes are immersed in the electrolyte about 6 cm apart. About 95% of the Hg is electrodeposited after 15 hours of electrolysis at a voltage of 4 to 6 volts and a current of 0.3 to 0.5 ampere. When the electrolysis is completed, the electrolyte is neutralized with NH_4OH, using phenol red as an indicator. Before the current is interrupted, the gold foil is removed from the solution, and rinsed with distilled water and alcohol. The electrical contacts are then removed and the gold foil is dried and reweighed to obtain the chemical yeild. The foil is sealed in a plastic bag and the $^{197-203}Hg$ activity measured by gamma-ray spectrometry. The use of a sample of 10 ml places the detection limit at 0.01 ng/ml.

Mercury Procedure III

Brune and Jirlow (1967) have developed a method for separating radiomercury from neutron activated fresh water samples based on the isotopic exchange technique reported by Kim and Silverman (1965). Water samples are neutron irradiated in polyethylene containers in a frozen state for 3 to 13 hours at a flux of about 2×10^{12} n/cm^2/sec. After the irradiation, the water samples are transferred to a distilling flask equipped with a reflux condenser. A 3:1 mixture of nitric and sulfuric acid is added and the sample refluxed for

about 20-40 minutes. The solution is cooled and then diluted with an equal volume of water; the pH is adjusted to 1 to 2 by addition of NH_4OH. The solution is transferred to an appropriate sized bottle and 0.5 gm of NH_4Br is added, followed by 0.2 ml of elemental stable Hg. The solution is vigorously stirred for 40 minutes to allow the radiomercury to exchange with the inactive Hg. The Hg is then separated on a sintered glass filter disk, washed with water and acetone, and then dissolved in 5 ml of concentrated nitric acid. The nitric acid is transferred to an appropriate container and $^{197-203}Hg$ is measured by gamma-ray spectrometry.

Mercury Procedure IV

Thatcher and Johnson (1971) have developed a precipitation method for measuring Hg in fresh waters by NAA. Water samples are placed in 20 ml quartz vials which are immediately sealed. The vial is neutron irradiated for 4 hours in a flux of 5×10^{12} n/cm^2/sec. One day after the irradiation the vial is opened, carrier Hg in nitric acid is added and the sample is allowed to stand one hour to desorb any radioactive Hg from the quartz. The sample is transferred to a polyethylene centrifuge tube, and ammonium persulfate is added to oxidize organo-mercurials. Sodium bisulfite is added to reduce excess persulfate and to dilute ^{24}Na and ^{35}S activities. Stannous chloride is next added to precipitate metallic mercury. The precipitate is centrifuged and counted for ^{197}Hg. Sensitivity is 0.05 µg/l but could be improved by simply activating the samples for a longer period of time.

The above four methods are capable of measuring only "total" mercury (inorganic plus organic forms), since the postirradiation digestion procedures should destroy any organically bound Hg. Although these four procedures have been developed especially for neutron activation analysis of Hg in fresh waters, there are numerous

postirradiation radiochemical separation schemes developed for other matrices which can be slightly modified to meet the needs of fresh water analyses. These procedures are contained in an excellent monogram by Roesmer (1970).

If Hg concentrations in fresh waters are too low to be measured in 1 to 30 ml volumes as described in the above procedures, preconcentration from large volumes (0.2 to 2 liters) of water is necessary. Lai and Weiss (1962) have described a very simple preconcentration procedure, which has subsequently been used at Battelle-Northwest Laboratory (Robertson, unpublished work, 1972) for preconcentration of Hg from seawater and should be applicable to fresh waters. A water sample (0.2 to 2 liters) is adjusted to a pH of 3.5 to 8, warmed to about 60°C, and 20 ml of a 1% solution of thionalid in acetone is added. The water samples are then chilled in a refrigerator, and the thionalid crystals (containing quantitatively cocrystallized Hg) are collected on membrane filters, washed with high purity water, and encapsulated in polyethylene vials for neutron activation analysis. ^{203}Hg can be directly measured in the neutron activated thionalid by Ge(Li) gamma-ray spectrometry several weeks after the irradiation.

Weiss and Crozier (1972) have described a method of preconcentrating Hg from seawater, which should be equally applicable to fresh waters. Mercury is separated from 500 ml of water by adding 1 mg of Cu carrier and coprecipitating the Hg with the Cu as sulfides by bubbling H_2S gas into the water for 30 seconds. The CuS precipitate is collected by membrane filtration, washed with high purity water, and encapsulated in a polyethylene vial for neutron activation. After the irradiation the $^{197-203}$Hg is radiochemically separated and measured by gamma-ray spectrometry.

Minczewski (1967), Mizuike (1965) and Joyner (1967) have described a number of group preconcentration procedures which should be adaptable for separating Hg from large volumes of water.

Cadmium Procedures

Landstrom and Wenner (1965) have outlined a post-irradiation radiochemical separation scheme for the measurement of Cd by neutron activation analysis, based on its anion exchange properties. However, the method is not generally applicable, because it lacks the sensitivity to measure environmental levels of Cd in only 10 to 30 ml of fresh waters. Therefore, it is necessary to preconcentrate the Cd from large volumes (0.2 to 2 liters) of water prior to the neutron activation step. Riley and Taylor (1968) and Goya and Lai (1967) have described a preconcentration method for Cd in seawater, based on adsorption onto Chelex-100 chelating resin, and this method should be adaptable to fresh waters. Other preconcentration schemes, as outlined by Minczewski (1967) and Mizuike (1965), should be rather easily adaptable for preconcentrating Cd from large volumes of fresh waters.

After the preconcentrates have been neutron activated, appropriate radiochemical separations as outlined by DeVoe (1960) may be used to isolate the radiocadmium further.

Silver Procedures

Kharkar *et al.* (1968), have measured Ag in river waters by NAA. Their procedure is to irradiate freeze-dried river salts with 25 mg of spec-pure Na_2CO_3 per 100 ml of river water added to provide enough salts with which to work conveniently. Several weeks after the irradiation, the salts are dissolved in 20 ml of 1 N HCl and silver carrier is added. Silver chloride is filtered off, rinsed with 0.1 N HCl, dissolved in a small volume of HNO_3, and then converted to an NH_4OH solution. The

solution is scavenged with $Fe(OH)_3$ to remove interferences, and the Ag is then precipitated with H_2S. The precipitate is dissolved in concentrated HNO_3 diluted with water, and the Ag is reprecipitated in dilute HCl. The AgCl precipitate is washed, dried, and counted for ^{110m}Ag.

The same thionalid preconcentration procedure (Lai and Weiss, 1962) described earlier for Hg can be used to isolate Ag from large volumes of fresh waters, to achieve the desired sensitivity. Silver can then be directly measured by counting the neutron activated thionalid crystals (containing quantitatively cocrystallized Ag) on a Ge(Li) diode detector or dual coincidence gamma-ray spectrometer. If further radiochemical purity is required, the method of Kharkar *et al.* (1968) can be used.

Arsenic Procedures

Arsenic can usually be measured in river or lake waters by the semi-INAA method of Tanner *et al.* (1972). However, in some natural waters, particularly precipitation, the As must be preconcentrated from large volumes of water. The As procedures described in the following section for seawater analyses are completely applicable for the determination of As in fresh waters.

Selenium Procedure I

For some natural waters the Se must be preconcentrated to achieve the necessary sensitivity. Kharkar *et al.* (1968) have developed a separation scheme for measuring Se in fresh waters. One hundred ml of freeze-dried river salts (containing 25 mg of spec-pure Na_2CO_3/100 ml of river water as a salt carrier) is neutron activated. Several weeks after the irradiation the sample is dissolved in 1 N HCl, carriers are added, AgCl is filtered off, the solution is made 6 N in HCl, and H_2S is added to precipitate Mo and Se sulfides. The Mo sulfides are dissolved away in dilute HNO_3, and the Se sulfide is purified by dissolving

in concentrated HNO_3, precipitating Se with SO_2, distilling off Se as the bromide, and finally reducing Se to the metal with $NaHSO_3$. The Se metal is filtered, washed, dried, and counted to measure ^{75}Se activity.

Selenium Procedure II

Weiss *et al.* (1971) have measured Se in melted samples of the Greenland ice sheet by NAA. One to 4 liter samples are acidified with HNO_3, evaporated to 1 ml, and neutron activated. After the irradiation the Se is radiochemically separated by repeated precipitations of the Se metal carrier. The final Se precipitate is filtered and beta-counted in a gas-flow proportional counter periodically to measure ^{81}Se and check its half-life. Yield is checked by counting carrier-free ^{75}Se added at the start of the procedure. Selenium concentrations as low as 5 ng/kg were measured.

Chromium Procedures

Chromium is frequently below the detection limit of INAA and must be preconcentrated from large volumes of fresh waters. The methods given for Cr measurements in the following section on seawater analysis are directly applicable for fresh waters.

Suspended Particulate Matter

The pollutant elements of concern contained on separated suspended particulate matter can be analyzed by post-irradiation radiochemical separations once the material has been properly digested. Refluxing in HNO_3-H_2SO_4 is normally a sufficient digestion procedure if silicious materials are not present. If large amounts of silica are present, HF must be added to the acid digest to volatilize the silica. Care must be exercised to ensure that volatile elements such as Hg are not lost during the digestion procedure.

Sea Water

A. Instrumental Analysis

Because of the tremendous amounts of ^{24}Na (15.4 hrs)

which are produced during neutron irradiation of sea water, INAA of sea water is applicable only for those elements in sea water which have relatively long half-life activation products. From Figure 4.5, it is obvious that none of the short-lived neutron activation products of interest could possibly be measured instrumentally, since the ^{24}Na, ^{38}Cl, ^{42}K and ^{82}Br are orders of magnitude more abundant and completely mask the gamma-ray contributions of the activation products of interest. For the INAA of sea salts, the neutron irradiated samples are stored for about one month to allow these relatively short-lived interferring radionuclides to decay to tolerable concentrations.

Figure 4.6 shows that after about 24 days out of the reactor, the ^{24}Na has decayed to insignificant concentrations, and ^{82}Br (36 hrs) is now the major gamma-emitting interferring radionuclide. Bromine-82 decays with the emission of a large number of high energy gamma-rays which interfere with the measurement of the trace activation products of interest, and it is necessary to store the samples for another two weeks to allow the ^{82}Br to decay to tolerable levels. High concentrations of ^{35}S and ^{32}P remain in the samples, but they are pure beta emitters and can be tolerated in the gamma-ray spectra.

The month-old activated sea salts are then transferred into a constant counting geometry (small polyethylene vials or stainless steel planchets) and counted directly on a Ge(Li) diode detector or a dual coincidence gamma-ray spectrometer (Robertson *et al.*, 1968). The activated sea salt samples are encased in a thin (0.7 mm) lead container to attenuate the low energy bremsstrahlung radiation from the beta-emitters ^{35}S and ^{32}P.

Figure 4.7 illustrates a typical gamma-ray spectrum of neutron activated sea salts counted on a Ge(Li) detector 54 days after the neutron irradiation and demonstrates

Figure 4.5

RELATIVE CONCENTRATIONS OF INDUCED RADIONUCLIDES IN SEAWATER FOLLOWING AN INTEGRATED THERMAL NEUTRON EXPOSURE OF 5 x 10^{16} n/cm^2. EMPHASIZING THE PROBLEM OF MEASURING SHORT-LIVED ACTIVATION PRODUCTS IN THE PRESENCE OF HIGH CONCENTRATIONS OF SODIUM-24, BROMINE-82, POTASSIUM-42 AND SULFUR-35.

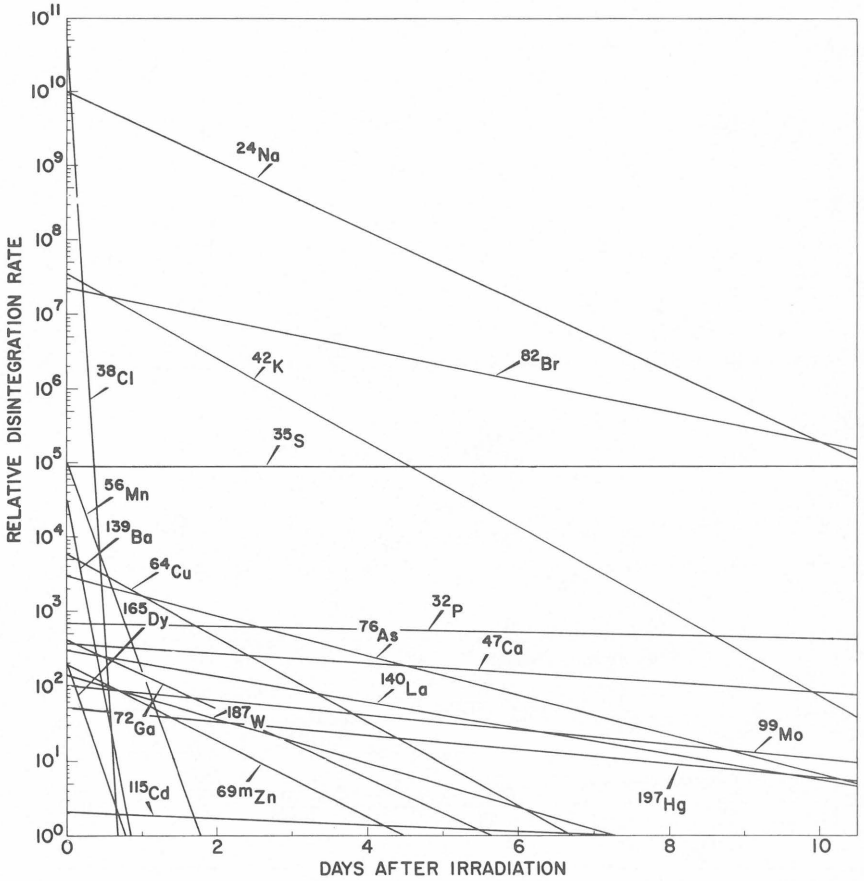


Figure 4.6

RELATIVE CONCENTRATIONS OF INDUCED RADIONUCLIDES IN SEAWATER FOLLOWING AN INTEGRATED THERMAL NEUTRON EXPOSURE OF 10^{18} n/cm^2. EMPHASIZING THE PROBLEM OF MEASURING INTERMEDIATE-AND LONG-LIVED ACTIVATION PRODUCTS IN THE PRESENCE OF HIGH CONCENTRATIONS OF SODIUM-24, BROMINE-82 AND SULFUR-35.

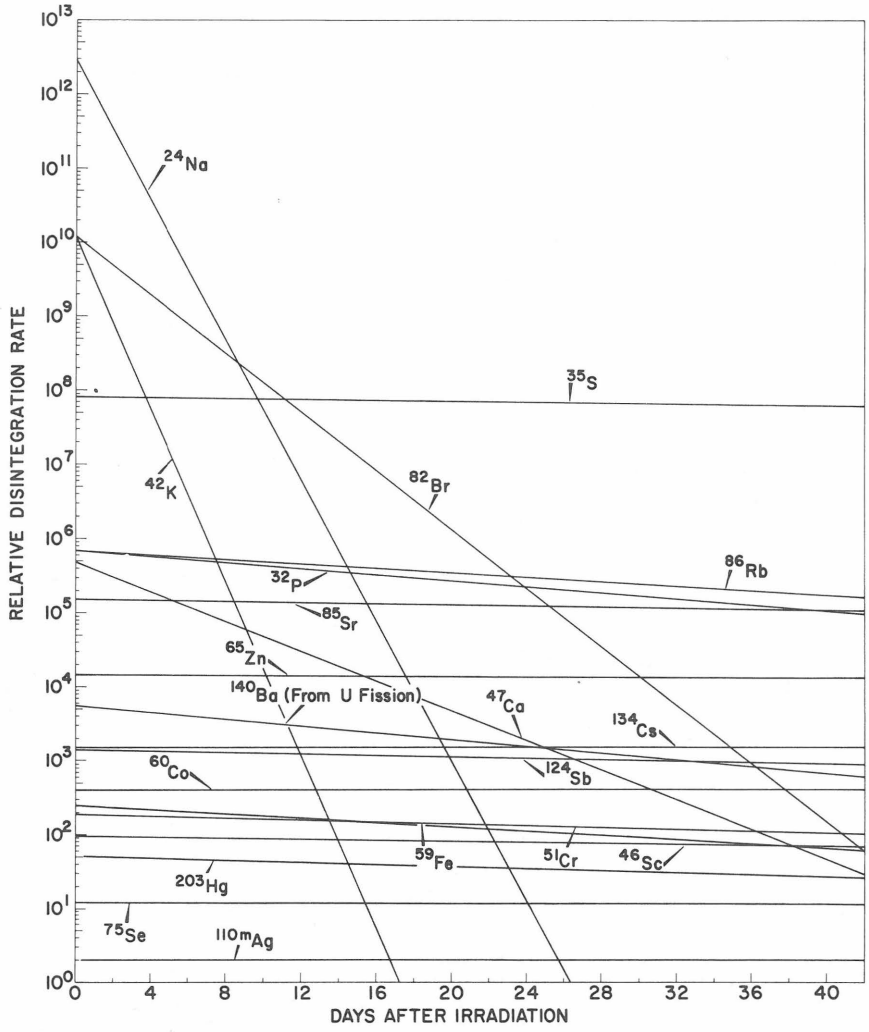


Figure 4.7 *GAMMA-RAY SPECTRUM OF NEUTRON ACTIVATED SEA WATER*

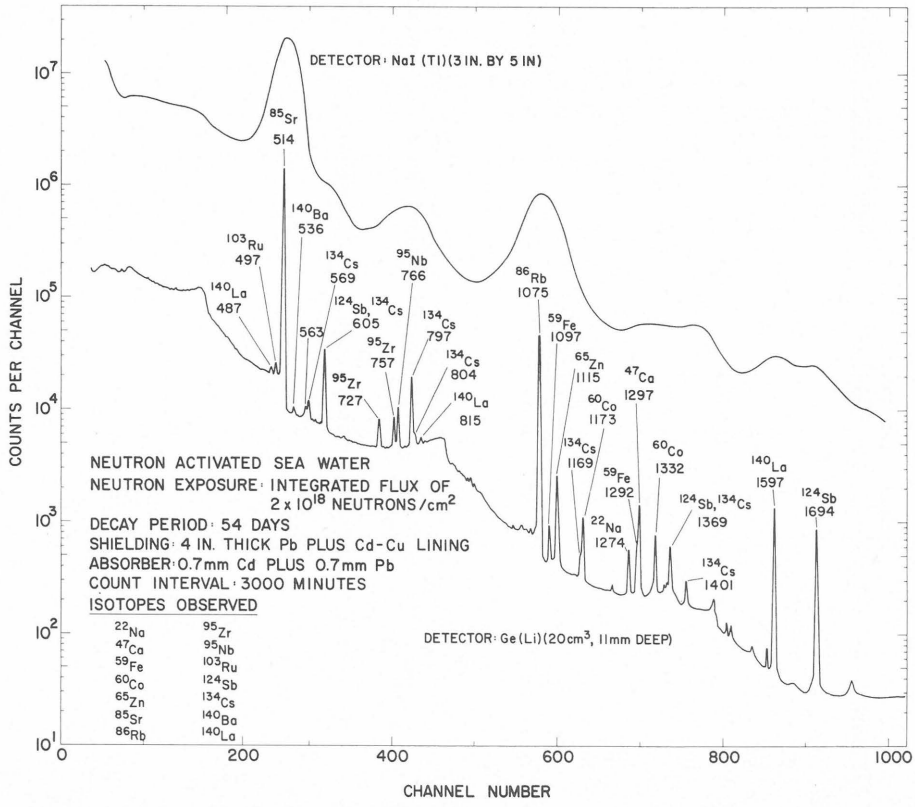


Table 4.2 Estimated Detection Limits for Instrumental Neutron Activation Analysis of Seawater*

Element	Typical Oceanic Concentration (µg/l)**	Detection Limit (µg/l)
Sr	8000	100
Rb	120	5
Fe	1-20(5)	1
Zn	0.5-10 (3)	0.2
U	3.3	0.1
Cs	0.3	0.003
Sb	0.2	0.005
Hg	0.01-0.3(?)	0.05
Co	0.001-0.1 (0.03)	0.001
Sc	$1\text{-}20 \times 10^{-4}$	1×10^{-4}

* 25 ml seawater; 24-hour irradiation at 10^{13} n/cm^2/sec; 40 days decay; 1000 minute count on 20 cc Ge(Li) diode detector.

** Estimated average concentrations in parentheses.

the tremendous resolution advantages of Ge(Li) detectors compared to NaI(Tl) crystals. It is apparent that the eight trace elements, Sr, Rb, Cs, U, Sb, Zn, Fe and Co, can be measured in sea salts by this technique. Only Sb and Zn are of immediate interest from an environmental pollution concern. Table 4.2 lists the trace elements in sea water which can normally be measured by INAA and compares the detection limits of this method with the actual oceanic concentrations of the elements observed in sea water. It is obvious that this method is sufficiently sensitive to measure any of the naturally existing concentrations of these elements observed in the oceans.

Mercury can be measured instrumentally in neutron activated sea salts by Ge(Li) diode spectrometry if the samples are irradiated in an extremely thermalized neutron flux. If the neutron flux contains an appreciable fast neutron component, large amounts of the pure beta-emitter ^{32}P are produced by a $^{35}Cl(n,\alpha)\ ^{32}P$ reaction. The bremsstrahlung activity from the ^{32}P (maximum beta particle energy 1709 KeV) interferes with the sensitive detection of the 279 KeV gamma ray from the ^{203}Hg neutron activation product. The Hanford plutonium production reactors, which have now all been closed down, were among the few nuclear reactors in the U. S. which possessed the necessary highly thermalized neutron fluxes. The Triga type of research nuclear reactors, now commonly available throughout the country, do not possess the required neutron flux characteristics necessary for the INAA of mercury in sea salts.

It is often desirable to remove suspended particulate matter from sea water for trace metal analyses, expecially in coastal or estuarine waters containing relatively high concentrations of biological and geological material. The techniques for removal and analysis are essentially the same as those described earlier in the section dealing with fresh waters.

Table 4.3 Estimated Minimum Detectable Concentrations of Pollutant Elements in Seawater by INAA and by NAA With Separations

Element	Typical Reported Concentrations in Open Ocean (Micrograms/Liter)	Minimum Detectable Concentrations (in micrograms/liter)	
		INAA*	NAA with Separations**
Hg	0.02-0.2	0.05	0.001
Cd	0.06-0.7	16,000	0.001
Ag	0.002-0.05	1.0	0.003
As	2-3	Not Possible	0.0001
Cu	0.5-2	Not Possible	0.002
Cr	0.02-0.6	0.3	0.003
Zn	0.5-10	0.2	0.01
Sn	0.02	Not Possible	9
Se	0.08	0.2	0.02
Sb	0.2	0.02	0.00003

* 25 ml seawater; 24 hour irradiation at 10^{13} n/cm^2/sec; 40 days decay; 1000 minute count on 20 cc Ge(Li) diode detector; based on 3σ above background -Compton contribution in peak areas.

** 500 ml seawater; elements chemically separated; 24 hour irradiation at 10^{13} n/cm^2/sec; 3 days decay; 500 minute count on a 20 cc Ge(Li) diode detector; based on twice background contribution in peak areas.

Of the pollutant elements of interest only Zn and Sb can be measured in seawater by INAA. However, if preconcentration or postirradiation separations are performed, neutron activation can be used to measure all of the pollutant elements of interest, except Pb and Sn. Table 4.3 lists the estimated minimum detectable concentrations of the potential pollutant trace metals of interest in seawater as measured by both INAA and NAA with separations from interfering radionuclides. These detection sensitivities are compared with the actual observed or estimated naturally occurring concentration ranges for these elements in the ocean. It is obvious that neutron activation with separations possesses the necessary sensitivity to measure all of these trace metals at naturally occurring concentrations well above their detection limits. Some of the necessary preconcentration and/or postirradiation chemical separation schemes permitting the measurement of these elements in seawater are given below.

B. Analyses of Elements Requiring Preconcentration or Postirradiation Separations

It should be pointed out that nearly quantitative losses of Hg from unacidified seawater onto polyethylene storage containers is a serious sampling problem. Both Hg^{++} and methyl Hg chloride are lost from solution by adsorption onto container walls. The Hg^{++} and methyl Hg chloride can be kept completely in solution for long storage periods by acidifying the seawater to a pH of about 1.5 with high purity HCl (Robertson, unpublished results, 1972, Battelle-Northwest).

Mercury Procedure I

The method of Ljunggren *et al.* (1971) described previously for analyses of Hg in fresh waters (see Mercury Procedure II in the section on fresh waters) has been utilized to measure Hg in seawater. The procedure is identical to

that for fresh waters, except that the neutron irradiated seawater samples must be stored in appropriate shielding until the very intense gamma-radiation resulting from high concentrations of ^{24}Na have decayed to workable levels. However, this procedure is not recommended, since the seawater irradiated in sealed quartz ampoules builds up tremendous pressures from radiolysis products, and the quartz ampoules are dangerous to handle.

Mercury Procedure II

Weiss and Crozier (1972) developed a preconcentration and post irradiation separation for measuring Hg in seawater by neutron activation analysis. To 500 ml of filtered, acidified (10 ml conc. HCl per liter of seawater) seawater was added 1 mg of Cu(II) carrier, and the Cu was precipitated as the sulfide by bubbling H_2S gas into the seawater for 30 seconds. The Hg in the seawater was coprecipitated with the CuS. The precipitate was collected on a membrane filter, rinsed with high purity water, and placed in a 15 ml polyethylene irradiation vial. The walls of the precipitation vessel were washed successively with 5, 3 and 2 ml volumes of nitric acid, and the washings were added to the irradiation vial. Blanks were prepared in an identical manner, except high purity water was used instead of seawater. The samples, blanks, and appropriate standards (10 ml in 15 ml irradiation vials) were neutron activated for 1 hour in a flux of 2×10^{12} $n/cm^2/sec$. After irradiation, the sample or blank was quantitatively transferred to a vessel that contained 1 ml of mercury carrier. The solution was brought to pH 1-2 with ammonia liquor, and 5 ml of tin (II) chloride were added. The precipitate which formed was isolated by centrifugation. The supernate was discarded, and the precipitate was dissolved in 5-10 ml of aqua regia. Copper carrier (1 ml) was added to the solution, the pH was adjusted as above, and precipitation with tin (II) was repeated. This precipitate was collected by filtration,

dissolved in aqua regia, and the solution was adjusted to pH 7 with ammonia liquor. Hydrogen sulfide gas was bubbled through the solution for 30 seconds. The sulfide precipitate was collected on a Millipore filter and counted. The irradiated standard Hg solutions, after addition of 1 ml of mercury carrier, were precipitated directly as the sulfide and counted.

The 77-Kev γ-ray of ^{197}Hg was measured one week after the irradiation with a NaI(Tl) detector coupled to a pulse-height analyzer.

After the radioactivity measurement the mercury sulfide was dissolved in 2 ml of aqua regia and diluted to 25 ml with water. Atomic absorption measurements of this solution were compared to that of a mercury standard to obtain the carrier yield. The yield is 81.1 $\pm$ 9.8% for 12 determinations and the sensitivity is 4 nanograms of Hg.

Mercury Procedure III

Robertson (unpublished data, 1972) has developed a very simple neutron activation procedure for measuring mercury in seawater. Seawater samples (stored at pH ≈1.5 to prevent Hg losses by adsorption onto container walls) of 200 to 1000 ml are adjusted to pH 3.5 to 8 with NH_4OH, warmed to about 60°C, and 20 ml of 1% thionalid is added. The seawater is chilled in a refrigerator, and the thionalid crystals (containing co-crystallized Hg) are collected on a membrane filter, washed with high purity water, and encapsulated in polyethylene irradiation vials. Blanks are prepared in an identical manner, using high purity water instead of sea water. The samples, blanks, and standards are neutron activated for 5 to 8 hours in a flux of about 5×10^{12} n/cm^2/sec. Several weeks after the irradiation the samples, blanks, and standards are transferred into new vials and counted in the same geometries on a Ge(Li) diode detector to measure ^{203}Hg. The recovery of Hg is quantitative, and the sensitivity is about 5 nanograms.

Silver Procedure I

The same thionalid co-cyrstallization procedure described above (Mercury Procedure III) is used for determining Ag in seawater, since Ag is also quantitatively co-crystallized from seawater by thionalid. Sensitivity can be improved by directly counting the samples on a dual coincidence gamma-ray spectrometer, described in the section on Gamma-Ray Spectrometry, to measure the multiple coincidence gamma-rays from ^{110m}Ag. The sensitivity is about 0.001 µg Ag/l.

Silver Procedure II

Schutz and Turekian (1965 a,b) have determined Ag in sea-water by neutron activating freeze-dried sea salts (from 75 ml of seawater) and appropriate Ag standards for two days in a flux greater than 10^{13} n/cm^2/sec. After several weeks the irradiated salts are dissolved in 25 ml of water and 1 ml of concentrated HCl, silver carrier is added, and the solution heated to coagulate the AgCl precipitate. The precipitate is centrifuged, washed with dilute nitric acid, treated with concentrated nitric acid, diluted with water, and then dissolved in ammonium hydroxide. Interferring radionuclides in the solution are scavenged twice by precipitation of ferric-hydroxide, and silver is finally dissolved in HNO_3 and precipitated as silver chloride. It is filtered, dried, and weighed as silver chloride. The precipitate is redissolved and ^{110m}Ag measured by gamma-ray spectrometry.

Arsenic Procedure I

Ray and Johnson (1972) report that serious losses (up to 70% in 1 week) occur by adsorption of As from seawater onto polyethylene storage containers, even when the seawater is acidified with 9 ml of concentrated HCl/1 of seawater. However, Robertson (1972 unpublished results), by using radio-arsenate and arsenite tracers, observed no losses of arsenic from seawater onto polyethylene container surfaces in both acidified and unacidified samples. E. A. Crecelius (1972 unpublished results, U. of Washington) has studied the loss of arsenic added to seawater and lake

water as As-74 arsenate. Tests were made with the water (1) acidified to a pH of 1 with HCl and stored at 20°C, (2) acidified and frozen, (3) unacidified and frozen, and (4) stored unacidified at 20°C. Losses of only 1-14% were found with polyethylene and pyrex containers over a two month period. Portman and Riley (1964) found polyethylene, pyrex, and soda glass absorb 6, 6 and 16%, respectively, of arsenate added to unacidified seawater.

Arsenic Procedure I

Robertson (1972 unpublished work) has measured arsenic in seawater by an $Fe(OH)_3$ coprecipitation technique. To 35 ml of seawater in 50 ml plastic conical centrifuge tubes is added 5 mg of Fe(III) carrier, followed by enough dilute NH_4OH to just precipitate the $Fe(OH)_3$. The precipitate is centrifuged, thoroughly washed twice with two 15 ml portions of high purity water to remove Na, transferred into small polyethylene irradiation vials, and slowly evaporated to dryness. Blanks are prepared in the same manner using 35 ml of high purity water instead of seawater. The samples, blanks, and standards are neutron irradiated for 5 to 8 hours in a flux of about 5×10^{12} n/cm^2/sec. Two or three days after the irradiation, the $Fe(OH)_3$ is dissolved in a 10:1 mixture of nitric-hydrochloric acids, transferred into new counting vials, and counted for 10-20 minutes each on a Ge(Li) diode detector to measure the ^{76}As 559 KeV gamma ray directly. Some ^{82}Br (554 KeV) and ^{122}Sb (565 KeV) are present, but are easily resolved by the Ge(Li) detector. The orchard leaf standards are counted on the Ge(Li) detector in an identical geometry to quantify the measurements. The yield is quantitative and the sensitivity is about 0.08 µg As/l.

Arsenic Procedure II

Ray and Johnson (1972) have used the thionalide preconcentration technique to separate As from seawater prior to neutron activation. To 250 ml of seawater is added 1 ml of 5% ascorbic acid, and the sample is heated just to boiling. The sample is allowed to cool for 10

minutes, and 0.5 ml of ascorbic acid is added and cooled to room temperature. Ten ml of 5N H_2SO_4 is added followed by 2 ml of 2% thionalid in acetone. Stir 5 minutes, wait 10 minutes, bring the solution just to boiling, and boil very slowly for 30 minutes to remove acetone. Remove from heat and cool overnight. Collect thionalide crystals (containing co-crystallized As) on membrane filters, rinse with 300 ml of deionized water and encapsule wet filter in a polyethylene vial. Blanks are prepared by using deionized water instead of seawater. The samples, blanks, and standards are neutron irradiated for 7 hours in a flux of 10^{12} n/cm^2/sec. One day after the irradiation, count the samples, blanks, and standards on a Ge(Li) diode detector to measure the ^{76}As 559 KeV gamma ray.

Copper Procedure I

Slowey and Hood (1971) have modified the procedure of Rona *et al.* (1962), for determining inorganic and organic forms of Cu in seawater. The seawater samples are oxidized for 5 minutes by reflux digestion with 10 ml each of 0.1N potassium persulfate and 0.4N H_2SO_4. The solution is cooled, adjusted to a pH of 5.5 to 6.5, and 2 ml of 2% sodium diethyldithiocarbamate in water is added and mixed. Twenty ml of chloroform is added and the solution shaken for 15 minutes to extract Cu. Three extractions are made, and the combined chloroform is scrubbed by shaking with high purity water. The metal carbamate salt is decomposed with three 15 ml portions of 1N HNO_3. The acid solution is evaporated to a small volume, transferred to 1.5 ml polyethylene vial, evaporated to dryness, heat-sealed, and neutron activated with appropriate standards for one hour in a flux of 2 x 10^{12} n/cm^2/sec. After neutron activation the copper is separated by sorption on Dowex 1x8 (200 mesh) anion exchange resin from 12N HCl. The resin is washed with 12N HCl and ^{64}Cu and ^{56}Mn are eluted together using 2.5N HCl. The ^{64}Cu and ^{56}Mn in the sample and standards are measured by gamma-ray spectrometry.

The method is nearly quantitative, and the sensitivity for Cu is 3×10^{-3} µg.

No other methods for measuring Cu in seawater by neutron activation analysis have been reported in the literature, but a convenient method for concentrating Cu from large volumes of seawater is by sorption on Chelex-100 chelating resin (Riley and Taylor, 1968). Copper is quantitatively retained on Chelex-100 at a pH of 7.6 and eluted with 2N HNO_3, and it may be possible to measure Cu without any further separation. If further radiochemical purity is required, a postirradiation separation employing a suitable solvent extraction procedure could be readily accomplished.

Selenium Procedures

The only method for measuring Se in seawater by neutron activation analysis is that reported by Schutz and Turekian (1965b). Seventy-five ml samples of seawater are freeze-dried, and the salts and appropriate standards are neutron activated for 2 days in a flux greater than 10^{13} n/cm^2/sec. Several weeks after the irradiation the salts are dissolved in dilute HCl, and Se carrier is added. The Se is then precipitated as the sulfide by bubbling H_2S gas into the solution. The precipitate is centrifuged, washed with distilled water, dissolved in HNO_3, and reprecipitated as Se metal by bubbling SO_2 into the solution. The Se metal is separated from large amounts of ^{35}S by placing the Se precipitate in a quartz tube with one end closed and heating the tube to drive the selenium out of the tube into a weighed pyrex counting vial. The Se is oxidized to SeO_2 with HNO_3, dried and weighed. The SeO_2 is then dissolved in water and brought to a standard volume for counting by gamma-ray spectrometry. Selenium concentrations of 0.05 to 0.1 µg/l were reported by using this method.

This method is rather tedious and time consuming, and perhaps a more convenient method could be easily developed.

Chau and Riley (1965) found that Se(IV) could be co-precipitated from seawater with $Fe(OH)_3$ with ≈95% efficiency in pH 4-6 range. Any other forms of Se present in seawater must be converted to Se(IV) for quantitative coprecipitation with $Fe(OH)_3$. This may be a convenient method for preconcentrating Se from large volumes of seawater (0.2 to 2 liters) prior to neutron activation. If postirradiation separations are necessary, precipitations with H_2S or SO_2 could be employed. Preconcentration of Se from 0.2 to 2 liters of seawater prior to neutron activation is recommended to give the necessary sensitivity and to eliminate the high radiation levels produced by neutron activation of sea salts.

Cadmium Procedure

No method for measuring Cd in seawater by neutron activation analyses has been reported in the literature. However, Cd could be preconcentrated from large volumes of seawater by the Chelex-100 method of Riley and Taylor (1968). Cadmium is quantitatively retained on Chelex-100 chelating resin at pH 7.6 and eluted with 2N HNO_3. The 2N HNO_3 could be evaporated, the residue neutron activated, and ^{115}Cd (54 hrs) separated by anion exchange from strong HCl solutions or by sulfide precipitations.

It may be that the thionalid preconcentration procedures described for Hg and Ag would be applicable for Cd under the proper pH conditions.

Chromium Procedures

Schutz and Turekian (1965b) have developed a method for measuring Cr in seawater by neutron activation analysis, but the method is tedious and is not recommended as routine. Stanford (1971) reported a method based on extraction of Cr(VI) by methyl isobutyl ketone after acidification with hydrochloric acid. For analysis of total Cr, any Cr(III) present is oxidized to Cr(VI) with potassium peroxydisulfate and a copper (II) catalyst. Fukai (1967) and Chuecas and Riley (1966) reported that

CR(III) could be preconcentrated from seawater by co-precipitation with $Fe(OH)_3$. Any Cr(VI) present must be reduced to Cr(III) with sodium sulfite in acid medium to measure total Cr concentrations. The methods of Stanfield (1971) and Fukai (1967) have both used the diverse chemistries of Cr(III) and Cr(VI) in their methods as a measure of the chemical species naturally present in seawater. After Cr has been preconcentrated from a large volume (0.5 to 2 liters) of seawater, the concentrate can be neutron activated and postirradiation separations as outlined by Pijck (1964) can be performed.

Marine Organisms

A. Instrumental Analysis

INAA is particularly applicable for the measurement of trace metals in marine organisms. Approximately 18 trace elements can be measured in most marine organisms by INAA, including the potential pollutant elements Hg, Ag, As, Sb, Zn and Se (Haller *et al.*, 1969; Morrison and Potter, 1972; Rancitelli *et al.*, 1969; Robertson *et al.*, 1972). Cadmium and chromium are present in most biological tissues at concentrations just under their INAA detection limits, but can be measured in some specimens when their concentrations exceed about 1 and 0.1 µg/gm dry weight, respectively. The ultimate sensitivities achieved by INAA for many elements are limited by the high concentrations of the pure beta-emitter ^{32}P (14 d) formed in the activated samples. The bremsstrahlung radiation produced from the ^{32}P creates an instrumental dead time problem when biological samples receive too great a neutron exposure.

In practice, the INAA of biological tissue samples consists of encapsulating a 10-1000 mg sample of dried tissue (fresh to dry weight ratios should be obtained) in a cleaned plastic irradiation vial, and neutron irradiating the sample and appropriate standards to an integral thermal neutron exposure of about 10^{17} n/cm^2. After the irradiation, the samples and standards are transferred

into standard counting geometries and counted on a Ge(Li) detector at optimum times following the irradiation to measure both short- and long-lived neutron activation products. Figures 4.8 and 4.9 show some typical gamma-ray spectra of neutron activated marine organism muscle tissue, illustrating the neutron activation products which can be measured after both a short and long storage period between the irradiation and counting.

As in the case of natural waters, two to four days out of the reactor the major neutron activation products are ^{24}Na and ^{82}Br, but normally high enough concentrations of ^{42}K and ^{76}As are present for accurate measurements. Some ^{64}Cu is also present, but since some short-lived radionuclides in activated biological tissue are also positron emitters, it is safer to separate the ^{64}Cu radiochemically. When cadmium concentrations in the biological tissue are over about 1 µg/gm dry weight, ^{115}Cd (54 hr) can be instrumentally measured from its 528 KeV gamma-ray or the 335 KeV gamma-ray from the ^{115m}In isomer.

After the samples have been out of the reactor for about two weeks, most of the ^{24}Na and much of the ^{82}Br have decayed to tolerable levels, and a new suite of long-lived neutron activation products can be instrumentally measured including Rb, Cs, Fe, Zn, Ag, Co, Cr, Hg, Se, Sb and Sc. Table 4.4 lists the elements which can usually be measured by INAA, and compares their detection limits by this method with the typical concentration ranges normally found in marine biological tissue. As shown in Table 4.4 the INAA detection limits for most of these elements are sufficiently low to permit their measurement in almost any type of biological matrix. Cadmium, Ag, Cr, and Sb are frequently present in organisms at concentrations near or just below their INAA detection limits, and if necessary, postirradiation

Figure 4.8

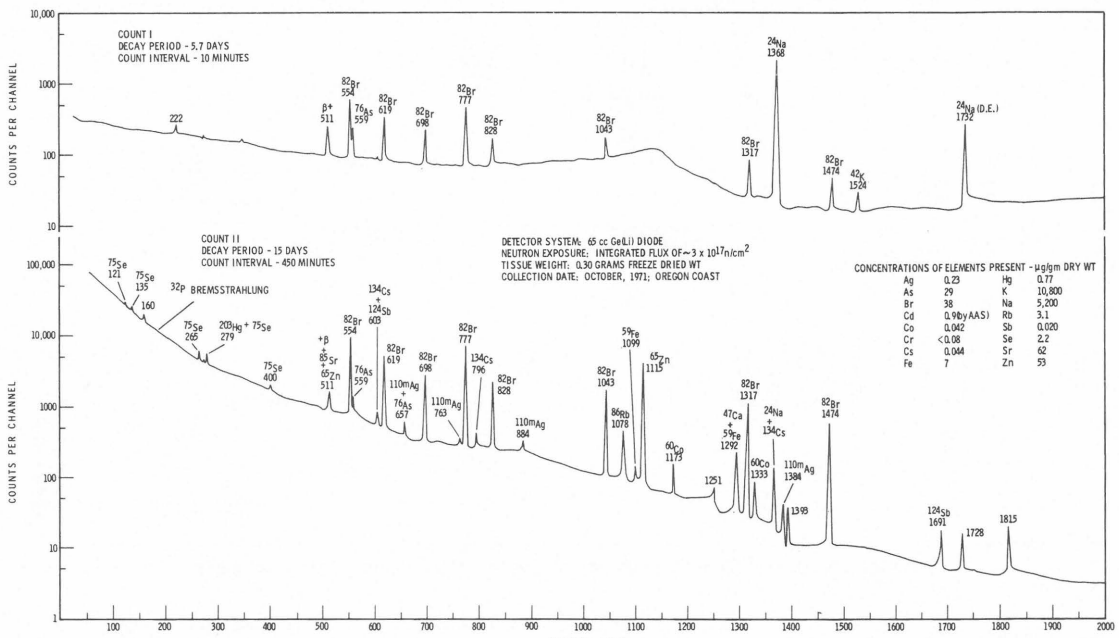

GAMMA-RAY SPECTRUM OF NEUTRON ACTIVATED PINK SHRIMP TAIL MUSCLE TISSUE (IDOE 63)
COUNT I
DECAY PERIOD - 5.7 DAYS
COUNT INTERVAL - 10 MINUTES
COUNT II
DECAY PERIOD - 15 DAYS
COUNT INTERVAL - 450 MINUTES
DETECTOR SYSTEM: 65 cc Ge(Li) DIODE
NEUTRON EXPOSURE: INTEGRATED FLUX OF ~ 3 x 10^17 n/cm^2
TISSUE WEIGHT: 0.30 GRAMS FREEZE DRIED WT
COLLECTION DATE: OCTOBER, 1971; OREGON COAST
CONCENTRATIONS OF ELEMENTS PRESENT - µg/gm DRY WT
Ag 0.23
As 29
Br 38
Cd 0.9(by AAS)
Co 0.042
Cr <0.08
Cs 0.044
Fe 7
Hg 0.17
K 10,800
Na 5,200
Rb 3.1
Sb 0.020
Se 2.2
Sr 62
Zn 53
COUNTS PER CHANNEL
ENERGY (KeV)

Figure 4.9

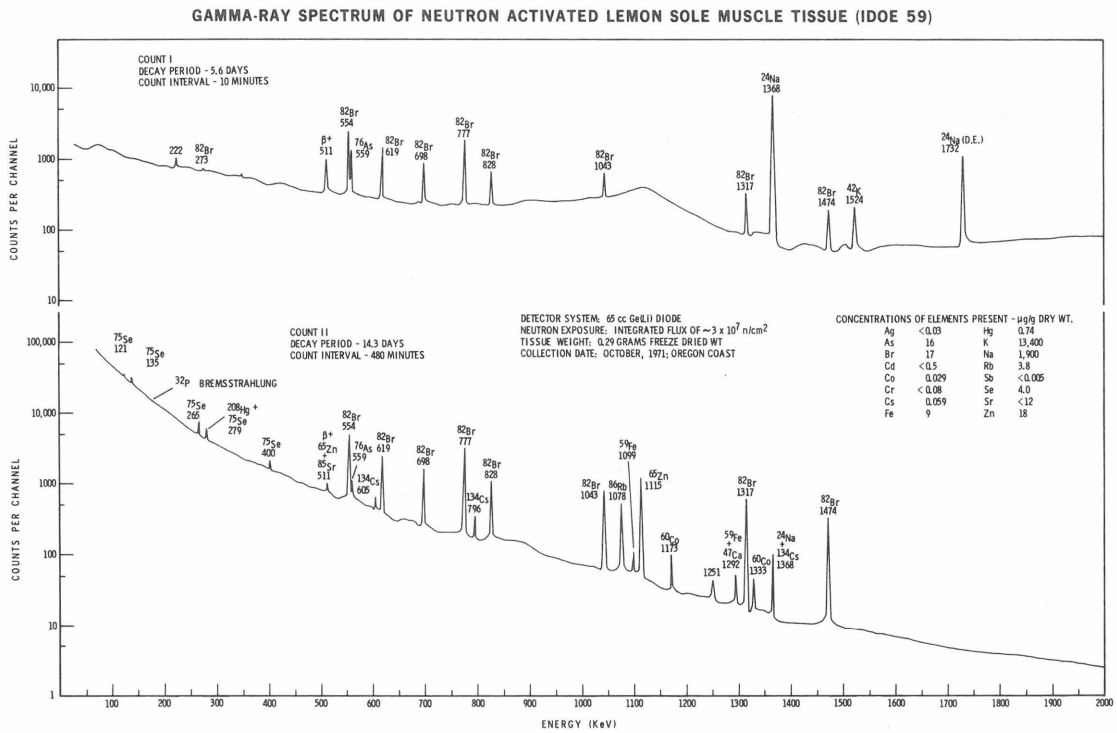

GAMMA-RAY SPECTRUM OF NEUTRON ACTIVATED LEMON SOLE MUSCLE TISSUE (IDOE 59)
COUNT I
DECAY PERIOD - 5.6 DAYS
COUNT INTERVAL - 10 MINUTES
COUNT II
DECAY PERIOD - 14.3 DAYS
COUNT INTERVAL - 480 MINUTES
DETECTOR SYSTEM: 65 cc Ge(Li) DIODE
NEUTRON EXPOSURE: INTEGRATED FLUX OF ~3 x 10^7 n/cm^2
TISSUE WEIGHT: 0.29 GRAMS FREEZE DRIED WT
COLLECTION DATE: OCTOBER, 1971; OREGON COAST
CONCENTRATIONS OF ELEMENTS PRESENT - µg/g DRY WT.
Ag <0.03
As 16
Br 17
Cd <0.5
Co 0.029
Cr <0.08
Cs 0.059
Fe 9
Hg 0.74
K 13,400
Na 1,900
Rb 3.8
Sb <0.005
Se 4.0
Sr <12
Zn 18
COUNTS PER CHANNEL
ENERGY (KeV)
BREMSSTRAHLUNG
B+ 511
32P
75Se 121
75Se 135
75Se 265
208Hg + 75Se 279
75Se 400
65Zn + 85Sr 511
76As 559
82Br 554
82Br 619
82Br 698
82Br 777
82Br 828
82Br 1043
82Br 1317
82Br 1474
134Cs 605
134Cs 796
86Rb 1078
59Fe 1099
65Zn 1115
60Co 1173
59Fe + 47Ca 1292
1251
60Co 1333
24Na + 134Cs 1368
24Na 1368
24Na (D.E.) 1732
42K 1524
222
82Br 273

Table 4.4 Estimated Detection Limits of Instrumental Neutron Activation Analysis of Biological Material (μg/g Dry Tissue)

Element	*Typical Concentration in Marine Organisms	Detection Limit**
Na	500-1500	0.05
K	1000-30,000	20
Rb	0.5-8	1
Cs	0.2-0.4	0.0001†
Fe	1-500	2
Zn	10-200	0.5
Br	10-200	0.05
As	<1-50	0.3
Cd	<0.1-10	1
Ag	<0.001-5	0.001†
Co	0.001-0.5	0.001†
Cr	<0.02-1	0.02
Hg	0.05-5	0.02
Se	0.5-50	0.05
Sb	0.0001-0.05	0.0001†
Sc	0.00001-0.002	0.00005†

* Typical Ranges in various tissues, with the low ranges usually associated with muscle and high ranges with liver.

** 300 mg of freeze-dried tissue; integral thermal neutron exposure of 3×10^{17} n/cm^2; 20 min. and 1000 min. counts after decay periods of 3 to 5 days and 20 to 30 days, respectively; 20 to 40 cc Ge(Li) diode detection, except where specified.

† Detection limits obtained by dual, cincidence NaI(Tl) spectrometry. Detection limits obtained by Ge(Li) spectrometry for these five elements are approximately 5- to 10-fold higher.

radiochemical separations of their neutron activation products can be performed by rather simple group chemical separations.

B. Analysis of Elements Requiring Preconcentration or Postirradiation Separations

The only elements which cannot routinely be measured in marine biological tissue by INAA are Cd, Cr, Cu and frequently Ag and Sb. However, these elements can be easily measured by neutron activation followed by radiochemical separations.

Cadmium Procedures

Many procedures for determining Cd in biological tissue have been reported: Lieberman and Kramer (1970); Das and de Vries (1971); Livingston *et al.* (1967); Ljunggren *et al* (1971); Edgington and Lucas (1970; 1971); Samsahl *et al.* (1968); Morrison and Potter (1972).

Biological tissues have been prepared for neutron irradiation in various ways. Some investigators irradiated fresh material, others freeze-dried or ashed samples. Sample decomposition after irradiation has generally been by digestion with H_2SO_4, with $K_2S_2O_8$ or HNO_3 added to speed dissolution. The samples ashed prior to irradiation are dissolved in concentrated HCl.

One Cd separation scheme uses the extraction with dithizone/chloroform solution as the key separation step (Lieberman and Kramer, 1970). Following $H_2SO_4/K_2S_2O_8$ digestion of the samples, the pH is adjusted to 1 to 2, and gross radioactive interferences are extracted into the dithizone layer while Cd stays in aqueous layer. Subsequently, the pH is adjusted to 13 to 14 and Cd is then extracted into the dithizone layer. Yields are determined by adding ^{109}Cd spike to each acid digestion sample.

Four other separation schemes (Morrison and Potter, 1972; Livingston *et al.*, 1967; Edgington and Lucas, 1970, 1971; Samsahl *et al.*, 1968) use anion exchange as the key to Cd separation. Cadmium forms strong chloro-complexes which

persist until very low HCl concentrations and the complexes are firmly bound to Dowex 1 or 2, or Amberlite IRA-400 anion exchange resins in the Cl form. The great majority of other elements and radioactivities can be eluted from the columns before the Cd. The Cd activity has been measured both on the resin directly and following elution and subsequent precipitation. Samsahl *et al.* (1968) and Bowen (1967) use several precipitation and dissolution steps involving CdS to remove Cd from interfering isotopes.

Copper Procedure I

Although the 511 KeV positron annihilation radiation from ^{64}Cu can usually be detected instrumentally in neutron activated biological tissue, other short-lived radionuclides, including ^{24}Na, are present which also contribute to positron annihilation radiation. Therefore, the ^{64}Cu should be radiochemically separated from interfering radionuclides, principally ^{24}Na. Four general separation schemes have been devised for Cu based on the insolubility of its sulfide, the solvent extractability of its organic chelates, its cation exchange behavior and its electrodeposition properties.

A rapid and simple method developed by Hahn *et al.* (1968), consists of digesting the biological tissue in nitric acid, dissolving the residue in 4 ml of 1N nitric acid, transferring the solution to a separatory funnel containing 2 ml of 0.1N HCl, and extracting ^{64}Cu with 5 ml of 0.01% dithizone in CCl_4 by shaking for 2 minutes. The CCl_4 is transferred to a polyethylene counting vial, and ^{64}Cu determined by gamma-ray spectrometry.

Copper Procedure II

Bird *et al.* (1969), have measured Cu in biological tissue by NAA after removal of ^{24}Na by retention on hydrated antimony pentoxide (HAP). The neutron activated tissue

sample is digested in fuming HNO_3-H_2SO_4, 0.1 mg of Cu carrier is added, and the solution is cooled and passed through a 0.5 gm column of HAP that had been preconditioned with 9N HNO_3. The column effluent (and 9N HNO_3 rinses) is collected and counted to measure ^{64}Cu. Some ^{54}Mn is present, but can be manually stripped from the gamma-ray spectra.

Copper Procedure III

Merlini *et al.* (1971), measure Cu in biological tissue by NAA after separating ^{64}Cu on columns of cuprous chloride or cupric sulfide. The irradiated tissue sample is digested in hot HNO_3-HF and brought to 6M $HClO_4$ or 1M H_2SO_4 and the solution is passed through a 7 mm diameter by 30 mm long column of cuprous chloride or cupric sulfide. The column is rinsed with 15 ml of the same acid and counted directly by gamma-ray spectrometry. Copper is retained on the column, while most interfering radionuclides are eluted. Only Au, Hg, Sb, Se, Br, W and I are wholly or partially retained on the column, but do not prevent the measurement of the 511 KeV ^{64}Cu activity.

Copper Procedure IV

Battistone and Lofberg (1970) quantitatively determine Cu in biological tissues by NAA utilizing an electrodeposition technique. The activated samples are digested in HNO_3 in the presence of Cu carrier, and the digest is taken just to dryness. The residue is dissolved in 1 ml of concentrated HNO_3 and rinsed into a 25 ml polyethylene beaker with 3 to 5 ml portions of the electroplating solution (10% sodium tartarate, 1% hydrazine hydrochloride, 1% sodium hydroxide, and 0.5% urea, prepared weekly). The Cu is electrodeposited onto cylindrical platinum electrodes at 2.5 volts and an initial current of 2.75 amperes for 45 minutes while stirred with a Teflon-coated magnetic stirring bar. The electrodes are

removed while the current is still on and rinsed with 95% ethyl alcohol. The electrodes are then directly counted by gamma-ray spectrometry, or the ^{64}Cu is dissolved off with several ml of 8N HNO_3 which is then counted.

Silver Procedures

Silver can be conveniently separated from neutron activated biological tissue by the method of Merlini *et al.* (1971). After digestion of the irradiated tissue in hot HNO_3-HF, the solution is brought to 6M $HClO_4$ and passed through a 7 mm diameter by 30 mm long column of cuprous chloride. Over 99% of the Ag is retained on the column, while interfering radionuclides are eluted with 15 ml of the same acid. The column can then be counted directly to measure the ^{110m}Ag by gamma-ray spectrometry. Only Cu, Au, Se, W, I, Hg, Pa, Sn and Sb are wholly or partially retained on the column, but do not interfere with the measurement of ^{110}Ag. Bromine-82, which is also retained on the column, would normally interfere, but the digestion step with HNO_3 should volatizile most of the Br. Once the biological tissue sample has been adequately digested by acid dissolution, the same Ag separations as used for seawater can also be employed here.

Antimony Procedure

Antimony can be determined by INAA in most biological tissue by measuring the 1696 KeV gamma-ray from ^{124}Sb. The sensitivity for measuring Sb is not limited due to interferences from other neutron activation products, but is limited by the dead-time to the Ge(Li) gamma-ray spectrometer caused by the high levels of the ^{32}P bremsstrahlung. Greater sensitivity could be achieved for Sb if a larger tissue sample were neutron irradiated for a longer time, and the activated tissue sample was encased in some material, such as 0.7 mm thick Pb, to attenuate the ^{32}P bremsstrahlung.

An alternative approach would be to use the rapid separation scheme of Merlini *et al.* (1971), whereby

$^{122-124}Sb$ is retained on a cuprous chloride column from 1M H_2SO_4.

Chromium Procedures

After digestion of neutron activated biological tissue samples, Cr may be separated by the solvent extraction procedure described in the section on seawater analysis.

Bowen (1964) has also developed a separation scheme for measuring Cr in biological tissue by NAA. After digestion of the irradiated samples in HNO_3-H_2SO_4 with Cr carrier added, and subsequently with $HClO_4$, the Cr is distilled into 10 ml of water from a $HClO_4$-H_2SO_4 solution to which 0.1 gm of NaCl had been added. The distillation with 2 more portions of NaCl is repeated. The distillate is next transferred to a separatory funnel and shaken with an equal volume of methyl isobutyl ketone, a few drops of concentrated HCl being added to improve the extraction. The aqueous layer is discarded, and the organic layer is washed with 2N HCl and then shaken twice with 10 ml portions of NH_4Cl. The NH_4Cl solutions are scavenged with $Fe(OH)_3$. The supernate is made acidic with acetic acid and treated with 2 ml of barium acetate. The $BaCrO_4$ precipitate is coagulated by boiling, centrifuged, dissolved in hot HCl, and reprecipitated at pH 6. The precipitate is washed twice with water, dried, weighed to determine yield, and ^{51}Cr is measured by gamma-ray spectrometry.

The above procedure is quite lengthy and Meloni *et al.* (1969) have measured Cr in biological tissue by removing interfering ^{24}Na and ^{32}P. Tissue samples are digested in HNO_3, and the solution is made 1N in HNO_3 and passed through a column of hydrated antimony pentoxide (HAP) to retain ^{24}Na. The effluent (and 1N HNO_3 rinse) is evaporated to about 10 ml and is then passed through a small column of SnO_2 which retains the ^{32}P. The

effluent (and 1N HNO_3 rinse) is treated with $KClO_3$ to oxidize Cr(VI), and the solution is passed through a small column of MnO_2 to retain Cr. The MnO_2 is filtered, dried, and counted directly to measure ^{51}Cr by gamma-ray spectrometry.

Sediments

A. Instrumental Analysis

Of the various analytical methods available for the determination of elemental concentrations in geological materials, neutron activation analysis is one of the most sensitive, selective, and reliable. INAA, by utilizing high resolution Ge(Li) detectors, permits the nondestructive measurement of 25 to 30 elemental constituents in geological material, including marine and fresh water sediments (Brunfelt and Steinness, 1966; Gordon *et al.*, 1968; Robertson *et al.*, 1972). However, most of the elements which can be measured in sediments by INAA are mainly of a geochemical interest, and of the pollutant metals of concern, only As, Sb and Cr can be measured routinely. Mercury and zinc can be measured instrumentally utilizing a special coincidence-anticoincidence Ge(Li) detector system (Cooper and Perkins, 1972). Utilizing Ge(Li) diode spectrometry and group radiochemical separations, the pollutant elements of interest can be measured (Filby *et al.*, 1970; Morrison *et al.*, 1969; Peterson *et al.*, 1969).

The general scheme for measuring trace elements in sediments by INAA is to weigh dried sediments (10 to 1000 mg) into cleaned plastic irradiation vials, heat-seal the vials, and neutron irradiate the samples, together with appropriate elemental standards encapsulated in an identical geometry to the samples. Elements with short-lived neutron activation products (2.8 minutes to several hours) are measured by irradiating the samples in a rabbit facility for several minutes at a thermal neutron flux of

1 to 50 x 10^{12} n/cm^2/sec and then counting the samples directly on a Ge(Li) diode detector for 5 to 10 minutes. Elemental standards are identically activated and counted to quantify the concentrations of the elements of interest in the samples. To determine those elements with intermediate half-life activation products (several hours to several days), the identical sediment sample used for the rabbit irradiation, or an aliquot of the same sample, plus elemental standards are neutron irradiated to an integral thermal neutron exposure of about 10^{17} n/cm^2. The samples and standards are then transferred into new counting containers and counted for 10 to 30 minutes on a Ge(Li) diode detector after 2 to 24 hours decay time. For the measurement of elements with long-lived activation products (several days to several years) the samples and standards are recounted 5 to 15 days later for 60 to 200 minutes. Some typical gamma-ray spectra of neutron activated deep sea red clay obtained by this method are shown in Figure 4.10. From the rabbit irradiations and short count it is possible to measure the elements Al, V, Mn, Na and Cl. Two to three hours after the long irradiation, the short-lived ^{28}Al and ^{52}V have decayed, and it is possible to measure Na, K, Mn, Cl, Ba and Dy. After a decay period of about 5 days the elements which can be measured are Na, K, Sc, Cr, Fe, Co, As, Br, Ba, Ca, La, Ce, Sm, Yb, Lu, Hf and Th (from ^{233}Pa).
The detection sensitivities for these elements which are obtained by INAA are dependent upon the composition of the sediment sample. For example, evaporite types of sediments having high Na and Br concentrations would create interferences which would limit the detection sensitivities of a number of elements. On the other hand, very pure calcareous sediments would permit more sensitive trace element analysis because of lower concentrations of interfering elements.

In Table 4.5, estimates of the detection limits for measuring trace elements in typical coastal or deep sea marine sediments by INAA are shown. The detection limits for most of the elements shown in Table 4.5 were derived by extrapolating from the INAA detection limits for these elements in the standard geological reference materials W-1 and G-2 (Gordon *et al.*, 1968). These standard rocks have trace element concentration ranges somewhat similar to those observed in marine sediments, and this comparison should give detection limits for marine sediments that are reliable to within an order of magnitude. As indicated in Table 4.5, INAA possesses the necessary sensitivity to measure all of the pollutant elements of interest instrumentally, except Ag, Cd, Cu, Se, Zn and frequently, Hg. The following section outlines methods for the post-irradiation separations of the activation products of these elements to give the desired sensitivity for their measurements.

B. Analysis of Elements Requiring Preconcentration or Post-irradiation Separations

The only elements of pollution concern which cannot normally be measured in sediments at natural background levels by INAA include Ag, Cd, Cu, Se, Zn and sometimes Hg. Radiochemical separation schemes are given below for these elements.

Mercury Procedures

One important point regardless of the type of post-irradiation treatment is that Hg may be lost in drying the sediments--either by freeze drying or oven drying. Preferably, the Hg analysis should be made on the wet sediment, and the wet-dry weight ratio determined on a separate aliquot of the sediment.

M. H. Bothner (unpublished data, University of Washington, 1972) has used nondestructive INAA to determine Hg levels of 0.5-17 ppm in Bellingham Bay sediments, based on

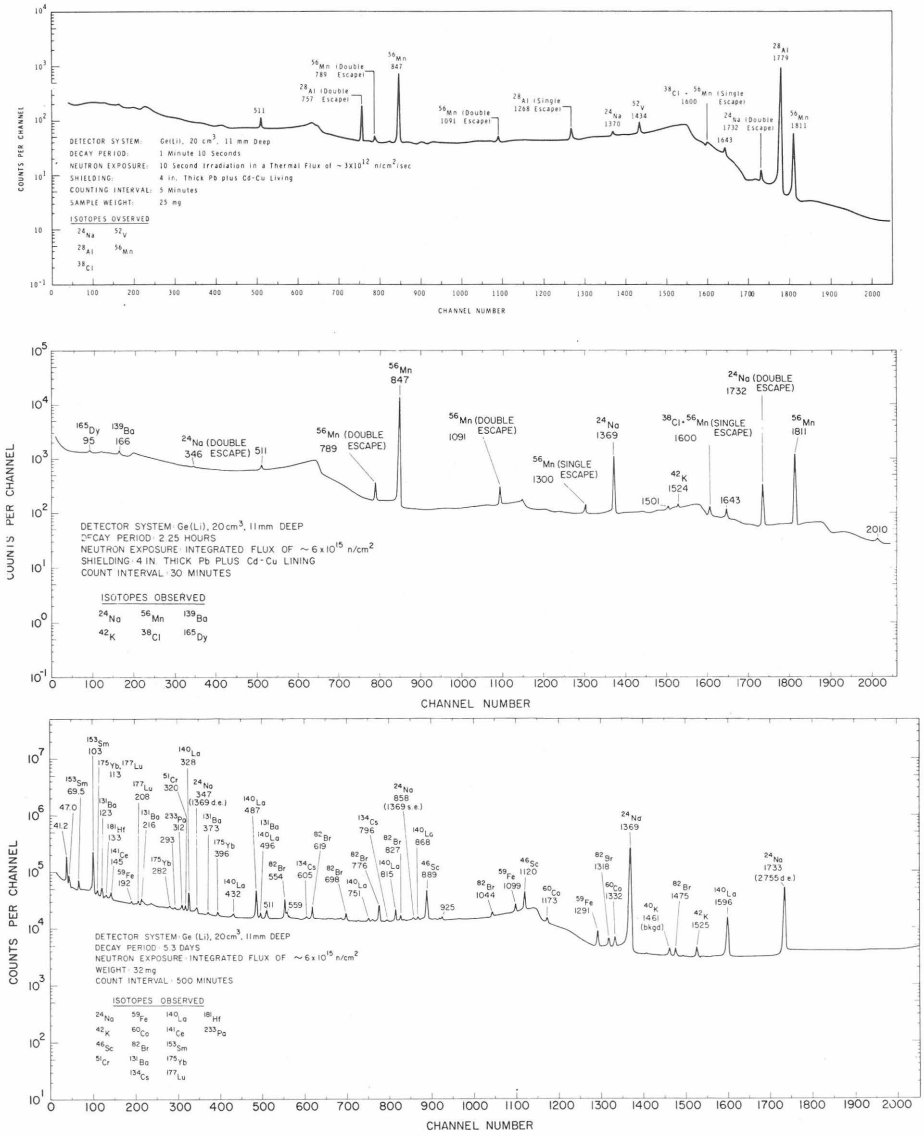


Figure 4.10 Gamma-Ray Spectra of Neutron Irradiated Deep Sea Red Clay Sediment after One Minute, Two Hours and Five Days Decay.

Table 4.5 Estimated Detection Limits for the INAA of Trace Elements in Marine Sediments

Element	Typical Concentration Ranges in Marine Sediments (μg/gm)	INAA Sensitivity* (μg/gm)
Ag	0.01-0.5	0.1
Al	10,000-90,000	10
As	2-20	1
Ba	60-8100	100
Ce	40-70	5
Cd	205	10
Co	1-200	0.1
Cr	10-200	0.8
Cs	0.3-15	0.2
Cu	10-700	1
Dy	0.2	0.1
Eu	0.2-10	0.05
Fe	20,000-60,000	200
Hf	0.1-18	0.1
Hg	0.05-3	0.5
K	3000-30,000	5000
La	2-60	2
Lu	0.2	0.1
Mn	100-10,000	10
Na	2000-40,000	100
Pb	10-200	---
Rb	1-100	1**
Sb	0.5-15	0.1
Sc	0.2-30	0.02
Se	0.1-1	2
Sm	0.5-30	0.3
Sn	0.5-15	500
Sr	200-2000	100**
Ta	0.03-3	0.01
Tb	0.1-7	0.1
Th	0.3-10	0.08
V	10-500	10
Yb	1	0.3
Zn	5-4000	5**
Zr	100-400	70

* 100 to 800 mg of dried sediment; samples irradiated at optimum intervals ranging from 1 minute to 6 hours in a flux of 10^{11} to 10^{13} n/cm^2/sec and counted at optimum intervals after the irradiation for 1 minute to 200 minutes; Ge(Li) diode detectors, 20 cc to 60 cc volumes.

** Determined by counting on a coincidence-anticoincidence shielded Ge(Li) gamma-ray spectrometer (Cooper and Perkins, 1972).

measuring the 279 KeV ^{203}Hg gamma ray. However, most sediments will have Hg levels at or below the lower limit of detection by the INAA ^{203}Hg method. Increased sensitivity is achieved by counting the 77 KeV gamma rays from ^{197}Hg. However, because of the relatively high concentrations of ^{24}Na activity produced by irradiating marine sediments, the ^{197}Hg must be chemically separated following the irradiation.

The main problem of postirradiation separations of Hg from neutron activated sediments is the dissolution of the samples. Ehmann and Lovering (1967) and Landstrom *et al.* (1969) point out that fusion of the sediments with Na_2O_2 or Na_2CO_3 is to be avoided because of loss of Hg before equilibration with the carrier is achieved. The most recommended digestion procedure is with HNO_3-H_2SO_4 in a Bethge apparatus or similar glass container, closed to prevent loss of Hg. However, this method will not dissolve siliceous sediments, and digestion with HF must be performed in addition to acid leaching; care being taken not to volatilize Hg during the process.

Following the digestion there is no uniform procedure for the Hg separation, but Ehmann and Lovering (1967), Kennedy *et al.* (1971), and Landstrom *et al.* (1969) use a key ion exchange step, taking advantage of the strong affinity of Dowex 1 or 2 for chloro-complexes of Hg to bind Hg to the resin while eluting most interfering isotopes. The Hg is subsequently eluted and precipitated as HgS for counting (Ehmann and Lovering, 1967) or electroplated onto Au or Pt foils (Pillay *et al.*, 1971), Landstrom *et al.*, 1969, and Ljunggren *et al.*, 1972). An extremely useful summary of extraction, coprecipitation and ion exchange behavior of Hg in different acid solutions is in Roesmer (1970).

Cadmium Separations

Once the sediment sample is dissolved by appropriate acid digestion, the general Cd separation procedures

outlined in the section on analysis of marine organisms can be used.

Landstrom *et al.* (1969), Morrison *et al.* (1969), and Peterson *et al.* (1969) have outlined sequential group chemical separation schemes for measuring a large number of elements, including Cd, in geological material by NAA. The methods of Morrison *et al.* and Peterson *et al.* are the same: After digestion of the sample by H_2SO_4-HF the residue is dissolved in 8N HCl and passed through a column of hydrated antimony pentoxide (HAP) to remove ^{24}Na. The effluent (and 8N HCl rinse) is passed through a column of Dowex 1x8 anion exchange resin which is then washed with 8N HCl. The resin, containing sorbed radiocadmium and radionuclides of Zn, Sb, Re, Ir and Au, is counted directly on a Ge(Li) detector to measure ^{115}Cd (54 hr).

Landstrom *et al.* (1969) treats the irradiated sediment with H_2SO_4-HBr to distill off the volatile bromides of Hg, Sn, Sb, As, and Se. The distillate is then digested with HF to complete the dissolution of siliceous material, and the residue is finally dissolved in HCl and passed through two columns of Dowex 2 anion exchange resins in the sulfate and hydroxide forms under various acid concentrations. The Cd is subsequently sorbed onto a column of Dowex 2 anion exchange resin in the sulfate form for counting of the ^{115}Cd by gamma-ray spectrometry.

Copper and Silver Separations

After dissolution of the neutron irradiated sediment by appropriate acid digestion the Cu and Ag can be separated by the procedures outlined in the sections on analyses of marine organisms and natural waters.

Selenium Separations

Case *et al.* (1969) described a group chemical separation method for determining Se in geological material by NAA. The irradiated sample is dissolved by H_2SO_4-HF digestion in the presence of carriers and made 6N

in HCl and 18N in H_2SO_4. The Se is distilled by dropwise addition of HBr at 220°C, using N_2 carrier gas. The distilled Se is precipitated as the sulfide, which is then dissolved in HCl-HNO_3 and counted by gamma-ray spectrometry to measure ^{75}Se. If further radiochemical purity is required, the procedures given in the sections on natural water analyses could be employed.

Akaiwa (1969), Greenland (1967), and Kiesl (1969) have reported radiochemical separation schemes for measuring Se in meteorites and Wiersma and Lee (1971) and Chau and Riley (1965) described nonradioactive determinations of Se in fresh water and marine sediments. Any of the procedures could be readily adapted to the measurement of Se in marine sediments by NAA.

Zinc Procedures

Zinc cannot routinely be measured in marine sediments, because the 1115 KeV gamma-ray from its neutron activation product ^{65}Zn is swamped by the high concentrations of ^{46}Sc, which has a major gamma ray of 1120 KeV. Therefore, to measure ^{65}Zn in neutron activated sediments, it needs only to be separated from ^{46}Sc. The sequential separation schemes developed by Morrison *et al.* (1969) and Peterson *et al.* (1969) can be used for this purpose. The procedure is exactly the same as that described earlier for measuring Cd in sediments.

Atmospheric Particulates

A. Instrumental Analysis

The contamination of the oceans by deposition of anthropogenic aerosols from polluted atmospheres is of real concern (see Duce *et al.*, 1972, and Robertson *et al.*, 1972 for examples). It is essential that the atmospheric concentrations of metal pollutants be monitored especially at coastal locations so that estimates of the air-to-sea transfer rates of these aerosols can be made. For the multielement analysis of atmospheric particulates collected on filter media, no analytical method can match INAA

for sensitivity and selectivity. Up to 33 elements can be measured in urban air by this nondestructive technique of selecting optimum neutron irradiation and counting times for the detection and measurement of neutron activation products ranging in half-life from 2.3 minutes to 5.3 years (Dams *et al.*, 1970; Dudey *et al.*, 1969; Gordon, 1971; Tuttle, 1971; Zoller and Gordon, 1970).

Plans for collection and preparation of samples for INAA are outlined in detail in the chapter dealing with transport processes. After collection, the tared filters are reweighed to determine the total weight of solids collected, and the filter or a known fraction of it is removed and encapsulated in a polyethylene irradiation vial. The filters and appropriate standards are then neutron activated for predetermined time intervals for the measurement of short- and long-lived neutron activation products.

A typical procedure for the analysis of elements forming short-lived activation products is to irradiate the filter sample for 5 minutes in a rabbit facility at a thermal neutron flux of about 1 to 50 x 10^{12} n/cm^2/sec, wait 2 to 10 minutes after the irradiation, and then count the sample for 5 to 10 minutes on a Ge(Li) diode detector. For the measurement of elements forming intermediate and long-lived radionuclides, the same sample or a portion of the same filter, plus elemental standards, are irradiated for 5 to 8 hours at a thermal neutron flux of 1 to 50 x 10^{12} n/cm^2/sec, and the samples and standards are then transferred into new counting containers and counted on a Ge(Li) diode detector for about 10 to 30 minutes when 1 to 3 days out of the reactor, and for about 60 to 200 minutes when 10 to 30 days out of the reactor.

The typical gamma-ray spectra which are obtained under these conditions are illustrated in Figure 4.11. In this particular aerosol sample collected in Cleveland, Ohio,

it is possible to measure from the short count the trace elements Al, Mg, Ti, V, Cu, Cl, Ba, Mn, and Br. From the intermediate count it is possible to measure Na, K, As, Br, Sb, Sm, La, U, and Cd and from the long count the elements Cu, Fe, Co, Zn, Se, Sb, Cr, Cs, Ce, Eu, Yb, Hf, Hg, and Th. The actual concentrations of these elements in this particular filter are given in Table 4.6. This filter contained an unusually high concentration of Sb, which was obviously released from some particular industry located in the sampling vicinity.

The detection sensitivities achieved by INAA for multi-element analysis of aerosols are not fixed values, but are dependent to a large degree upon the composition of the sample. For example, the relatively high concentration of Sb on the Cleveland air filter shown in Figure 4.11 limited the sensitivity of those elements forming neutron activation products with gamma-rays of lower energy than the main ^{124}Sb gamma rays at 603 and 1691 KeV. The Compton scattering from these and other ^{124}Sb gamma rays produced a relatively high "background" for the measurement of other gamma rays of lower energies. Nevertheless, estimates of the detection sensitivities of the elements which can be measured by INAA are useful and give one a feel for the excellent sensitivities which are obtainable by this technique.

An estimation of the minimum detectable concentrations of 37 elements in urban aerosols as measured by INAA is given in Table 4.7. The sensitivities obtainable ($\mu g/m^3$) in nonurban aerosols are even greater than those shown in Table 4.7, since larger volumes of cleaner air (lesser interelement interferences) can be sampled. The INAA method possesses the sensitivity to measure directly all of the metal pollutants of interest, except Cd, Pb, and sometimes Ag, Cu, and Hg. Cadmium, Cu, Ag and Hg can be measured by neutron activation followed by radiochemical separation of their activation products. Although

Figure 4.11 **GAMMA-RAY SPECTRUM OF NEUTRON ACTIVATED CLEVELAND AIR FILTER**

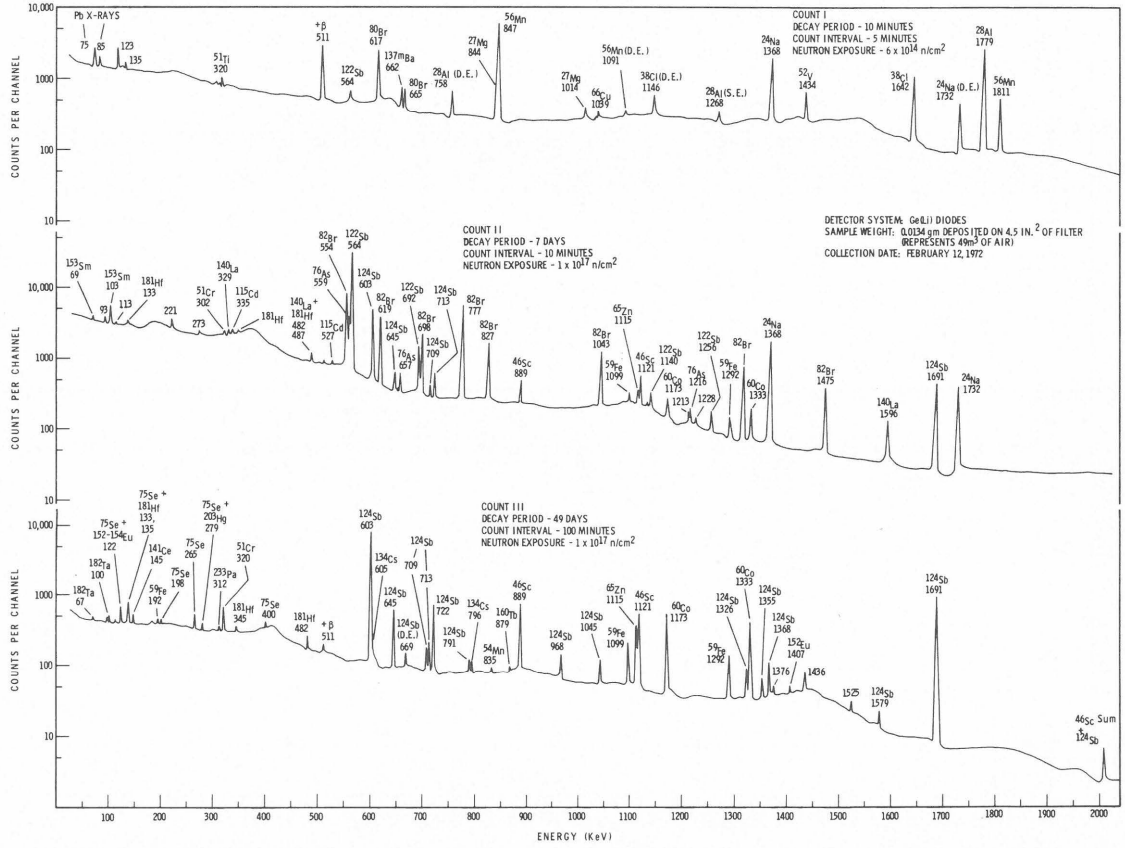


Table 4.6 Elemental Concentrations in Cleveland Air Filters February 12, 1972

Element	ng/m^3 Air	µg/gm Particulate
Al	9890	36600
As	316	1170
Br	513	1900
Cl	9450	34900
Co	32.7	121
Cr	96	360
Eu	0.075	0.28
Fe	9750	36100
Hf	1.61	5.96
Hg	<0.03	<0.1
La	7.2	27
Mg	2190	8100
Mn	277	1030
Na	10460	38700
Sb	503	1860
Sc	2.41	8.92
Se	27.4	101
Sm	1.20	4.44
Ta	0.66	2.4
Tb	0.21	0.78
Th	0.72	2.7
Ti	630	2330
V	17.8	65.9
Zn	1040	3850

Table 4.7 Sensitivities for Determination of Trace Elements in Aerosols by INAA (After Dams, *et al.* 1970)

Element	Decay Time Before Counting	Detection Limit* (μg)	Minimum Detectable Concentration In Urban Air (μg/m³, 24-Hr. Sample)†
Al	3 minutes	0.04	0.008
S	"	25.0	5.0
Ca	"	1.0	0.2
Ti	"	0.2	0.04
V	"	0.001	0.002
Cu	"	0.1	0.02
Na	15 minutes	0.2	0.04
Mg	"	3.0	0.6
Cl	"	0.5	0.1
Mn	"	0.003	0.0006
Br	"	0.02	0.004
In	"	0.0002	0.00004
I	"	0.1	0.02
K	20-30 hours	0.075	0.0075
Cu	"	0.05	0.005
Zn	"	0.2	0.02
Br	"	0.025	0.0025
As	"	0.04	0.004
Ga	"	0.01	0.001
Sb	"	0.03	0.003
La	"	0.002	0.0002
Sm	"	0.00005	0.000005
Eu	"	0.0001	0.00001
W	"	0.005	0.0005
Au	"	0.001	0.0001
Sc	20-30 days	0.003	0.000004
Cr	"	0.02	0.00025
Fe	"	1.5	0.02
Co	"	0.002	0.000025
Ni	"	1.5	0.02
Zn	"	0.1	0.001
Se	"	0.01	0.0001
Ag	"	0.1	0.001
Sb	"	0.08	0.001
Ce	"	0.02	0.00025
Hg	"	0.01	0.0001
Th	"	0.003	0.00004

* Air filters (20 x 25 cm, or aliquots thereof) neutron irradiated at optimum intervals, ranging from 5 min. to 5 hrs. at fluxes of 2-15x10^{12} n/cm^2/sec; counted for 7 to 70 min at optimum intervals ranging from 3 min to 20-30 days after the irradiation; 30 cc Ge(Li) detector.

† 24 hr sampling period at air sampling rate of 12 liters/min-cm^2.

Pb and Sn are insensitive to neutron activation analysis, it is worth pointing out that another nuclear method, x-ray fluorescence spectrometry, is convenient for the non-destructive analysis of Pb in aerosol samples.

B. Analysis of Elements Requiring Preconcentration or Post-Irradiation Separations

Occasionally, Cd, Cu, Ag, and Hg are present on air filters in concentrations too low to measure by INAA, and radiochemical separations of the neutron activated filters are necessary to separate interfering radionuclides. Since the particulate material collected on aerosol filters resembles geological and/or biological matrices, the radiochemical separations for these elements from irradiated air filters are the same as those described for geological and/or biological materials. The filters and collected aerosols are dissolved by digestion in H_2SO_4 or HNO_3-HF. Once the samples are in solution, the previously described radiochemical separations for the elements of interest may be performed.

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Appendix

PERTINENT NUCLEAR DATA RELATING TO THE MEASUREMENT OF THE TRACE METALS IN MARINE ENVIRONMENT SAMPLES BY INSTRUMENTAL AND NON-INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS

ELEMENT	TARGET ISOTOPE	ISOTOPIC ABUNDANCE, %	PRODUCT NUCLIDE	HALF-LIFE	THERMAL NEUTRON CROSS SECTION (BARNS)	BEST GAMMA-RAY FOR MEASUREMENT (KeV)	NUMBER OF GAMMA PER 1000 DECAYS	ASSOCIATED GAMMA-RAYS KeV (γ's/1000 DISINTEG.)	POSSIBLE INTERFERRING RADIONUCLIDES IN MEASUREMENT OF BEST GAMMA-RAY (KeV)
Ag	Ag-107	51.82	Ag-108	2.4m	35	633	17	433(4.5), 614(2.6)	Nd-151(629), Nd-149(630), Dy-165(633)
	Ag-109	48.18	Ag-110m	260d	3	657	960	764(230), 884(710), 937(317), 1384(211)	As-76(657)
Al	Al-27	100	Al-28	2.3m	0.23	1779	1000	NONE	Nd-151(1776)
As	As-75	100	As-76	26.5h	4.5	559	440	657(66)	Br-82(554), Nd-149(556), Eu-152m(562)
Au	Au-197	100	Au-198	64.8h	98.8	411	960	676(9.7), 1088(1.94)	Eu-152(416), Ir-192(417)
Ba	Ba-130	0.101	Ba-131	11.6d	8.8	496	450	124(200), 216(190), 373(130)	Cd-115(492), Ru-103(497)
	Ba-138	71.66	Ba-139	83m	0.4	166	217		Th-233(163), Gd-161(165), Th-233(169), Mg-27(170), Nd-151(171)
Br	Br-81	49.463	Br-82	35.5h	3.3	777	257	554(226), 619(133), 1044(90), 1317(87)	W-187(772), As-76(775), Eu-152(779)
Ce	Ce-140	88.48	Ce-141	32.5d	0.6	145	490	NONE	Ce-143(142), Fe-59(142), Yb-175(145), Eu-154(146), Eu-152(148), Te-131m(150)
Cd	Cd-114	28.86	Cd-115	2.3d	1.1	335	528	492(101), 528(264)	Te-131m(334), Fe-59(334), Tb-160(337)
	Cd-116	7.58	Cd-117	2.5h	1.4	273	310	314(160), 345(180), 434(13)	Br-82(273)
Cl	Cl-37	24.471	Cl-38	37.3m	0.56	1642	380	2167(470)	NONE
Co	Co-59	100	Co-60	5.24y	19	1332	1000	1173(1000)	NONE
Cr	Cr-50	4.31	Cr-51	27.8d	17	320	90	NONE	Ir-192(317), Lu-177(319), Nd-147(319), Os-193(321), Lu-177(321)
Cs	Cs-133	100	Cs-134	2.1y	28	795	880	475(150), 569(140), 605(980),	Te-131m(794)
Cu	Cu-63	69.09	Cu-64	12.9h	4.5	511	380	1345(5)	ALL POSITRON EMITTERS
	Cu-65	30.91	Cu-66	5.1m	2.3	1039	93	833(2.5)	Nd-151(1042), Ga-70(1042)
Dy	Dy-164	28.18	Dy-165	2.35h	700	362	10	280(5.7), 633(6.4),	Th-233(360), Gd-159(364),
Eu	Eu-151	47.82	Eu152m	9.3h	2800	841	111	122(50), 344(17), 963(93)	Mn-56(846), Te-129(845)
	Eu-151		Eu-152	12.4y	5900	122	294	344(285), 964(162), 1112(154),1408(229)	Yb-169(118), Se-75(121), Eu-154(123), Ba-131(124)
Fe	Fe-58	0.33	Fe-59	45d	1.2	1099	560	192(25), 1292(440)	Tb-160(1103)
Hf	Hf-180	35.24	Hf-181	43d	10	482	810	133(400), 136(60), 346(130)	Re-188(483), Eu-154(478), W-187(480), Ir-192(485), Re-188(485), La-140(487)
Hg	Hg-196	0.146	Hg-197	65h	880	77	180	191(10)	Pt-197(77)
	Hg-202	29.80	Hg-203	46.6d	4	279	820	NONE	Hg-197m(279), Se-75(280),Os-193(280), Yb-175(283), Ir-192(283)
In	In-113	4.28	In-114m	50d	8	190	161	558(35), 725(35)	Eu-154(188), Te-131m(189), Hg-197(191), Pt-197(191), Fe-59(192)
	In-115	95.72	In-116m	54m	154	1097	530	417(360), 1293(800)	NO SHORT-LIVED INTERFERENCES
K	K-41	6.88	K-42	12.4h	1.1	1525	180	313(1.8)	NONE
La	La-139	99.911	La-140	40.2h	8.9	1596	970	328(210), 487(460), 816(240)	Ga-72(1596)
Lu	Lu-176	2.59	Lu-177	6.7d	2100	208	75	113(44)	W-187(206), Lu-177m(208)
Mg	Mg-26	11.17	Mg-27	9.5m	0.03	1014	300	844(700)	Mo-101(1012), Nd-151(1016)
Mn	Mn-55	100	Mn-56	2.58h	13.3	847	990	1810(297), 2113(151)	Mg-27(844)
Na	Na-23	100	Na-24	15.0h	0.13	1369	1000	2754(1000)	NONE
Ni	Ni-58	67.88	Co-58	71d	0.1	810	990	511(300)	Ca-47(807), Eu-152(810)
	Ni-64	1.08	Ni-65	2.56h	1.5	1482	836	366(150), 1115(547)	Ge-77(1478), Mo-93m(1479), Nd-151(1486)
Rb	Rb-85	72.15	Rb-86	18.7d	0.9	1076	88	NONE	NONE
Sb	Sb-121	57.25	Sb-122	2.8d	6	564	663	692(33)	As-76(559), Cs-134(563), Eu-152(566)
	Sb-123	42.75	Sb-124	60.2d	3.3	1691	500	602(980), 723(98), 2091(69)	NONE
Sc	Sc-45	100	Sc-46	83.8d	13	889	1000	1121(1000)	Ag-110m(884), Ir-192(884)
Se	Se-74	0.87	Se-75	120d	30	264	630	121(176), 136(604), 279(264),400(126)	Yb-169(261), Cd-115(263), Ta-182(264) La-140(267)
Sm	Sm-152	26.72	Sm-153	47h	210	103	270	NO MAJOR	Ta-182(100)
Sr	Sr-84	0.56	Sr-85	65d	0.8	514	100	NO MAJOR	As-76(510), La-140(511), W-187(512), β^+ EMITTERS
Ta	Ta-181	99.988	Ta-182	115d	21	1221	278	100(142), 1121(350), 1189(166), 1230(117)	Ir-194(1219), As-76(1220)
Tb	Tb-159	100	Tb-160	72d	46	879	318	298(263), 962(126), 966(276),1178(166)	W-187(879), Ce-143(880)
Th[a]	Th-232	100	Th-233	22.1m	7.4	-	440	NO MAJOR	Nd-147(319), Ir-192(317), Cr-51(320)
			Pa-233	27.0d	-	312			
Ti	Ti-50	5.34	Ti-51	5.80m	0.14	320	955	608(15), 928(45)	Pt-199(317), Ru-105(317), Ta-182m(318), Nd-151(320)
U[b]	U-238	99.27	U-239	23.5m	-	-	120	106(230), 209(40), 278(140)	Gd-159(226), Lu-177m(228), Ta-182(229), Ce-143(232)
			Np-239	2.35d	2.7	228			
V	V-51	99.76	V-52	3.77m	4.9	1434	990	NONE	Ba-139(1430)
Yb	Yb-176	12.73	Yb-177	1.9h	7	150	168	121(17), 1079(47), 1120(10), 1241(26)	Te-131(150),Kr-85m(151),Sr-85m(151)
Zn	Zn-64	48.89	Zn-65	243d	0.46	1116	506	NO MAJOR	Te-129m(1112),Eu-152(1112),Ta-182(1113), Tb-160(1115), Sc-46(1121)
	Zn-68	18.57	Zn-69	14h	0.1	439	950	NONE	Nd-147(440), Os-193(441)

[a] THORIUM IS MEASURED BY COUNTING THE 27 DAY ^{233}Pa DAUGHTER OF ^{233}Th

[b] URANIUM IS MEASURED BY COUNTING THE 2.35 DAY ^{239}Np DAUGHTER OF ^{239}U

Spectrophotometric Analysis (Herbert L. Windom)

Introduction

Although many of the new techniques for the analysis of heavy metals require neutron activation capabilities many have been designed for the more commonly available colorimeter or atomic absorption spectrophotometer. In this section analytical techniques are described for several heavy metals (As, Cd, Co, Cu, Pb, Hg, Ni, Ag. and Zn) that utilize the latter instrumentation. While some of the techniques described have been in use by marine scientists for some time, and have therefore stood the test of time, most have been only recently developed. This section is divided into three parts dealing with the analysis of each of the metals in water, biological and sediment samples.

All procedures described are applicable to both marine and estuarine samples. In the case of water samples most of the techniques are salinity independent or have a correction for salinity built-in. Colorimetric analytical techniques are described for some metals in water only where existing atomic absorption techniques have proved inadequate. In this section it is assumed that the reader has a working knowledge of colorimetry and atomic absorption spectrophotometry.

Sea Water

The analysis of heavy metals in sea water requires different preconcentration and separation steps for almost every metal. The analytical techniques described below are therefore divided accordingly.

A. Arsenic

Arsenic may be concentrated from sea water by the co-cyrstallization with thionalide and determined photometrically as described by Portmann and Riley (1964). Since only a few samples may conveniently be prepared at a time, the method is too time consuming for a large number of samples.

The arsine generation method using atomic absorption with an argon-hydrogen entrained air flame has been evaluated. The procedure follows that described in the section on biological samples with the exception that a larger sample solution volume is used. Unfortunately, the larger volume of the system makes the sensitivity much poorer than that reported for biological samples. Attempts to concentrate the sample by absorbing the generated arsine gas into a dilute solution of silver nitrate were also unsuccessful. Further work is needed in order to develop this method. The cocrystallization and photometric technique as described by Portman and Riley (1964) appears to be the most suitable at present and is outlined below.

Reagents

(1) Ascorbic Acid 5% W/V
(2) Thionalide (thioglycollic-β-aminonaphthalide) 2% W/V in acetone.
(3) Sulphuric Acid (5N)
(4) Ascorbic Acid 0.1 m (prepare daily)
(5) Potassium Antimonyl Tartrate 0.274%
(6) Ammonium Molybdate 4.8%
(7) Mixed Reagent - 50 ml of 5N H_2SO_4 + 15 ml of ammonium molybdate, 5 ml potassium antimony tartrate, and 30 ml of 0.1 m ascorbic acid. Dilute to 125 ml. Reagant should be used within one hour of preparation.

Procedure

(1) To 1 L of filtered sea water in a 1 L Erlenmeyer flask, add 4 ml of 5% ascorbic acid.
(2) Cover with a watch glass and heat to boiling.
(3) Add an additional 2 ml of 5% ascorbic acid and cool to room temperature.
(4) While stirring the sample with a magnetic stirrer, add 10 ml of 5N H_2SO_4 and 7 ml of thionalide solution.
(5) Stir the solution five minutes to coagulate the precipitate.

(6) Allow to stand 10 minutes, then heat to a gentle boil for 30 minutes.

(7) Cool and stir to coagulate the precipitate.

(8) Allow to stand overnight.

(9) Filter off the precipitate with gentle suction using a 4.25 cm diameter Whatman No. 541 filter paper.

(10) Wash the precipitate and filter with double distilled water.

(11) Place the filter and precipitate in a 25 ml Erlenmeyer flask with glass stopper.

(12) Add 7.5 ml of conc. HNO_3, close the flask, and heat gently until the solution is pale yellow (24-36 hours).

(13) Evaporate the nitric acid at low temperature.

(14) When a yellow viscous liquid remains, pass CO_2 gas over the surface while heating.

(15) Continue the gas until the dense white fumes subside. This destroys any HNO_3 present.

(16) Cool the residue (a pale yellow solid) and add 1 ml of 1N H_2SO_4.

(17) Warm gently to dissolve and transfer to a 10 ml volumetric flask; add 2 ml of the mixed reagent and dilute to volume.

(18) Allow 30 minutes to elapse and measure the absorbance at 866 mμ using a 4 cm cell; compare with standards and blanks made up in 1 L of double distilled water and treated in the above manner.

C. Cadmium

Cadmium in sea water occurs at levels too low for direct measurement by colorimetry or by atomic absorption spectrophotometry. Atomic absorption analyses require enrichment of the species being sought from the original sample in order to reach concentrations above its limit of detection. Evaporation of sea water is lengthy, cumbersome, and the inevitable concentration of the salts present makes analysis difficult due to matrix interference. The extraction technique of Brooks *et al.* (1967) using ammonium pyrolidine

dicarbamate as a complexing agent may be useful if cadmium concentrations are several parts per billion. However, normal sea water levels are less than a part per billion, making this technique applicable only to enriched samples such as polluted estuarine waters or sediment pore waters.

Riley and Taylor (1968b) have described a method using a chelating ion exchange resin for concentration and the subsequent determination by atomic absorption. The method is also applicable to other trace metals (Co, Mn, Ni, Zn). It involves chelating the trace metals on Chelex 100 resin and eluting them with a small volume of acid. The procedure outlined below follows that described by Riley and Taylor (1968) with several modifications. The eluate from the column after being evaporated to dryness is dissolved in HCl rather than acetone. It was found that the addition of acetone or other miscible organic solvents produces a precipitate which clogs the aspiration assembly of the atomic absorption system. Also, if larger than 1 L samples are used, the ion exchange column should be backwashed occasionally to avoid resin compaction. Standards may be prepared by spiking sea water which has been stripped of trace metals by passage through a column of the chelating resin. A blank is prepared by processing stripped sea water in the absence of the spike. The absorption from the blank is subtracted from the absorption of the samples and standards. This will correct for absorption due to matrix effects of samples having similar salinity (within 10°/₀₀). Samples differing by more than 10°/₀₀ salinity should be treated individually.

The pH of the sea water sample must be adjusted to 7.8 $\pm$ 0.1 prior to analysis. The ion-exchange resin is pH selective, and the pH adjustment is critical if 100% recovery is to be obtained. The sample flow rate through the column must not exceed 5 ml/minute since the resin has a very slow

exchange rate. Replicate analyses of one large water sample have indicated a precision of ± 7% using this technique.

Reagents

(1) Chelex-100 ion exchange resin. 50-100 mesh.
(2) HNO_3 (2N) (127 ml conc. HNO_3/L).
(3) HCl (2N) (167 ml/L).

Procedure

(1) Wash a suitable aliquot of the resin with excess 2N HNO_3 three times. (1 ml/ml of resin). Approximately 10 ml of resin are needed per column.
(2) Wash the resin with double distilled H_2O and pack an ion exchange column (1 cm diameter) to a depth of 9.0 cm. To avoid air bubbles in the resin, pour a few ml of double distilled H_2O into the column, then pour the resin in a thick slurry. The water level in the column should never be allowed to drop below the resin bed level. This is best accomplished by connecting a piece of tygon tubing to the bottom of the column and looping it above the resin bed.
(3) Connect the ion exchange column to a reservoir and wash the resin with an additional 50 ml of H_2O.
(4) Allow the sea water sample, pH = 7.8, to flow through the column (5 ml/min). Check the flow rate occasionally with a stop watch and graduate cylinder. If more than 1 L of sample is used (usually 5L is used); the column should be backwashed every two hours with distilled deionized water.
(5) Wash the column with 250 ml H_2O and discard the wash.
(6) Elute the metals with 30 ml of 2N HNO_3 followed by 20 ml of 2N HCl and finally with 20 ml of double distilled water. Combine the eluants in a vycor flask.
(7) Evaporate the solution to dryness at low temperature and dissolve the residue with 1 ml of 2N HNO_3.
(8) Dilute the solution to 5 ml. If 5 L of sample was used a 1/5 dilution may be necessary for Ni and Zn.

C. Cobalt, Copper, Nickel, and Zinc

Cobalt has been determined in sea water by a method proposed by Weiss and Reed (1960). The metal is co-cyrstallized with α-nitroso-β-napthol and determined colorimetrically with nitroso-R-salt. However, the use of 50 to 100 L samples required by this technique limits its feasilbility. Cobalt may also be determined by atomic absorption after concentration by ion-exchange (Riley and Taylor, 1968b, as described under cadmium); however, the normal level found in sea water approaches the limit of detection of the method. The most feasible and easily accomplished analytical technique for Co appears to be the APDC/MIBK extraction (Brooks *et al.*, 1967) followed by atomic absorption spectrophotometry.

Copper has been determined by a dithizone-carbon tetrachloride extraction as described by Sandell (1955), but the complex is not very specific. Lengthy separation steps are necessary to isolate copper. Atomic absorption analysis after sample extraction with APDC-MIBK offers greater sensitivity without the necessity of separation (Brooks *et al.*, 1967). Cadmium, cobalt, copper, iron, nickel, and zinc may also be determined by the method if concentrations are above one part per billion. The trace metals are chelated and extracted from a 750 ml volume of water into a small volume of organic solvent. Standards are prepared by spiking water from which the trace metals have been previously extracted. The spiked samples are chelated and extracted following the same procedure as the samples. Replicate analyses indicate a precision of $\pm$ 12% for copper.

Nickel can be determined in sea water by extraction with dimethylgloxime (Kenter *et al.*, 1969). The method has a limit of detection of about 0.35 µg/l and is free from any salt effect. Cobalt and copper also form dimethylgloximine complexes but only at concentrations much greater than

those found in sea water. Nickel may be more easily determined by the atomic absorption - APDC/MIBK method with a precision of ± 12% (Brooks *et al.*, 1967), however at concentrations below 2.0 ppb, the precision drops substantially.

Zinc can be determined colorimetrically by a dithizone extraction method (Sandell, 1965), but the presence of other trace metals interfere, so that separation prior to analysis is required. Riley and Taylor (1968a) claim 100% retention of zinc on Chelex-100 ion-exchange resin. The zinc is eluted from the resin and determined by atomic absorption. Replicate analysis indicates a precision of ± 10% (see cadmium technique). The extraction method of (Brooks *et al.*, 1967) may also be useful, especially when other trace metals (Co, Cu, Fe, Ni) are of interest. The precision is about the same as for the above method. All glassware must be cleaned with scrutiny and the use of rubber in any apparatus must be avoided.

In summary, the most universally applicable technique for the analysis of Co, Cu, Ni and Zn is the APDC/MIBK-AA method. It has found general use in many labs and is the preferred method here. The following therefore describes this technique in detail.

Reagents

(1) Redistilled methy isobutyl ketone.

(2) APDC (1%) (prepared daily). Shake the 1% APDC (ammonium pyrolidine dicarbamate) solution with an equal volume of MIBK; separate and filter the aqueous phase through Whatman #5 filter paper. This cleans the APDC solution.

Procedure

(1) Carefully adjust the pH of the sample to between 4 and 5.

(2) Measure and pour 750 ml of sample into a polypropylene Erlenmeyer flask.

(3) Add 7 ml of 1% APDC and 35 ml of MIBK.

(4) Equilibrate the sample for 30 minutes on a shaker, then pour into a 1 L. separatory funnel.

(5) Separate the phases and put the organic phase into a plastic beaker and cover the beaker with parafilm.

(6) Add 20 ml of MIBK to the aqueous layer, shake for five minutes, separate and discard the organic phase.

(7) Homogenize the aqueous phase of all samples and measure the temperature of the homogenized sample.

(8) From a temperature-solubility chart (Table 4.8), determine the % of soluble MIBK in the homogenized sample.

(9) Separate the homogenized water into four 750 ml aliquots and spike with incremental amounts of trace metals. These serve as standards.

(10) Add 7 ml of 1% APDC to each spiked sample.

(11) Add 35 ml of MIBK less the volume found soluble from step i.

(12) Equilibrate the samples on a shaker and extract as before.

(13) Determine absorbance for standard and samples by AA.

(14) Prepare a calibration curve from the standards and determine the sample concentrations.

D. Lead

Lead in sea water can be determined by the method described by Riley and Taylor (1968b) if concentrations are about 0.5 µg/L. The sensitivity may be increased by using a larger sample volume (5-10 L). Upon analysis by atomic absorption, the absorbance observed is largely due to molecular absorption interferences. The absorbance due to lead is only a fraction of the total observed absorbance. Therefore, the precision of the method unfortunately is very poor at the concentrations normally found in sea water. For this reason the technique has application only in areas such as polluted estuaries.

E. Mercury

A colorimetric method for the determination of mercury involving a dithizone extraction is described by Sandell

Table 4.8 Temperature Solubility Chart

Temp. (°C)	% Solubility	Insoluble MIBK
0	2.63	15.27 ML
2	2.54	15.98
4	2.45	16.66
6	2.36	17.30
8	2.28	17.91
10	2.20	18.50
12	2.13	19.05
14	2.06	19.58
16	1.99	20.07
18	1.92	20.54
19	1.90	20.76
19.5	1.88	20.87
20	1.87	20.97
20.5	1.86	21.07
21	1.84	21.18
21.5	1.83	21.28
22	1.82	21.38
24	1.77	21.75
26	1.72	22.10
28	1.68	22.41
30	1.64	22.70

(1965). This method, however, lacks both specificity and sensitivity. Atomic absorption with an air-acetylene flame lacks the sensitivity needed for sea water analysis even after prior concentration. Recently, flameless atomic absorption has been established as a sensitive method for analysis of microgram and submicrogram amounts of mercury.

Kalb (1970) describes a method whereby mercury is amalgamated on silver foil and subsequently heated in an induction furnace, liberating the mercury which is swept into the instrument's optical path. However, matrix problems make reproducible results difficult to obtain. Liberating mercury from organic matter by permanganate oxidation has been described by Hatch and Ott (1968). The sample is subsequently reduced with a solution of sodium chloride/hydroxylamine sulfate/stannous sulfate. This one step reduction also suffers from irreproducibility. Uthe *et al.*, (1970) have devised a sophisticated reaction chamber which they claim gives more reproducible results than that of Hatch and Ott.

Chau and Saitoh (1970) described a method for mercury analysis of lake water which is also applicable to sea water samples as well. The method involves a dithizone-chloroform extraction and the subsequent back extraction into 5N HCl. The acidic solution is reduced with stannous chloride and vaporized by a peristaltic pump. Samples spiked with methyl and phenylmercuric compounds have been found to be quantitatively extracted with the dithizone solution. Interferences from other metallic ions are minimized by the addition of EDTA and hydroxylamine hydrochloride to the sample.

The absorption tube (16 x 2.5 cm) through which the mercury vapor passes is made of glass, has an inlet and outlet opening, and has quartz windows on either end. This tube may be placed in the optical path of any conventional atomic absorption system. The reaction chamber used is

a 125 ml gas washing bottle. Air from a peristaltic pump (1 L/min) is forced through the glass fritted tube inside the gas washing bottle. A drying tube containing magnesium perchlorate is placed between the reaction flask and the absorption tube to prevent water vapor from entering the absorption tube. This tube should be replaced or refilled after 12-15 analyses to avoid loss in sensitivity. The mercury vapor is recycled in a closed system until the absorbance reaches a maximum. The maximum absorbance is more easily determined when a recorder is connected to the AA system.

Standards may be prepared by spiking 40 ml of 2.5 N HCl with incremental amounts of mercury. To ascertain quantitive recovery several previously extracted samples may be spiked and re-extracted. A sample blank may be prepared similarly.

Reagents

(1) Dithizone-chloroform. Dissolve 6 mg of dithizone in 1 L $CHCl_3$. Remove any mercury present in this solution by extracting with an equal volume of 5N HCl.
(2) Disodium EDTA solution - 0.10 M.
(3) Hydroxylamine hydrochloride (50% W/V).
(4) Stannous chloride - 20% (W/V) in 6N HCl.
(5) HCL (5N) 417 ml. conc. HCl/L.

Procedure

(1) To 500 ml of filtered sea water (pH adjusted to 2 using dilute HCl), add 2 ml of hydroxylamine hydrochloride and 1 ml of EDTA.
(2) Add 25 ml of dithizone-chloroform solution and shake 20 minutes on a mechanical shaker.
(3) Allow the sample to stand 20 minutes, then separate the organic layer into a 60 ml separatory funnel.
(4) Add 20 ml of 5 N HCl and shake 10 minutes.
(5) Discard the organic layer, and transfer the acidic solution into a small beaker. Wash the funnel with 20 ml of redistilled water, and add the wash to the beaker.

(6) Pour the contents of the beaker into the reaction flask.

(7) Add 1 ml of $SnCl_2$ and close reaction flask quickly. Adjust pump speed to 1.0 L/min.

(8) Allow the absorbance to reach a maximum before opening the system.

F. Silver

Silver concentration in sea water is at the submicrogram per liter level. It must, therefore, be concentrated prior to analysis by conventional means. Riley and Taylor's (1968b) chelating ion exchange technique claims 100% retention of silver ion on the chelating resin. However, only 10% of the silver can be eluted from the resin. Attempts to digest the resin with $HClO_4$ and HNO_3 have been unsatisfactory.

Lai and Weiss (1962) have investigated the use of thionalide for the co-crystallization of 27 elements including silver. The crystals are dissolved with hot nitric acid and the organic matter destroyed. Silver is separated from interfering ions by extraction with tri-N-butyl thiophosphate. The organics present must then be destroyed with nitric and perchloric acids.

The subsequent determination of silver may be determined by two methods. Method I is based on the catalytic action of silver on the persulfate oxidation of manganous ion to permanganate as described by Underwood *et al.* (1952). This method is recommended for the determination of less than 0.25 µg of silver. Method II involves a photometric determination using p-dimethylaminobenzalrhodanine as described by Sandell and Newmayer (1951). This method is recommended for the determination of greater than 0.5 µg of silver. Method I is preferred due to its sensitivity. Standards may be prepared by running spiked samples through the colorimetric procedure only. A check on the efficiency of the co-cyrstallization may be accomplished by running standards through the entire procedure.

Reagents

(1) Thionalide solution (1% W/V in acetone).
(2) Acetic acid (conc.)
(3) Tri-N-butyl thiophosphate (1.5% W/V in CCl_4).
(4) Phosphoric acid (1:1).
(5) Manganous sulfate (0.006 M).
(6) Potassium persulfate (granular).

Procedure

(1) Adjust the pH of 1 L sea water sample to 3.5-4.0 with acetic acid.
(2) Heat the sample to 80° C, then add 20 ml of the thionalide solution.
(3) Allow the solution to cool at room temperature, then place in a refrigerator (5°C) overnight.
(4) Collect the crystals in a sintered glass funnel.
(5) Wash the crystals with a small volume of redistilled water and discard the wash.
(6) Dissolve the crystals with 75 ml of hot conc. HNO_3.
(7) Add 10 ml of conc. H_2SO_4 and boil until carbonization occurs.
(8) Add several 2 ml portions of 1:1 HNO_3-$HClO_4$ to clear the solution, then carefully evaporate to dryness.
(9) Dissolve the white inorganic residue with 20 ml of 6N HNO_3.
(10) Extract this solution four times with 10 ml portions of thiophosphate solution.
(11) Combine the extracts, and wash with 40 ml redistilled water.
(12) Evaporate the CCl_4 at room temperature, then wet ash the residue with 10 ml of HNO_3 and 3 ml of $HClO_4$.
(13) After complete evaporation of the acid, the residue is dissolved with 5 ml of redistilled water.
(14) To a 15 ml conical centrifuge tibe containing 1 ml of phosphoric acid solution and 1 ml of manganous sulfate solution, add the 5 ml sample.

(15) Adjust the total volume to 10 ml using redistilled water washing of the previous sample container and add 2.0 gm of potassium persulfate.

(16) Mix the solution and place all samples and standards simultaneously in a boiling water bath for 10 minutes.

(17) Simultaneously place samples and standards in an ice bath and allow to stand 20 minutes with occasional shaking.

(18) Centrifuge the samples and standards and place back in the ice bath until ready to determine their optical density (at 525 mμ).

(19) Avoid bubbles in the cell by keeping the tip of the transfer pipet below the surface of the liquid during delivery.

(20) Compare samples with standards.

Biological Samples

Metal concentrations in marine organisms are much higher than those in sea water therefore generally eliminating the necessity of preconcentration prior to analysis. However, other difficulties are encountered with biological samples that are not as severe in water samples.

The greatest problem in the chemical analysis of marine organisms is the lack of uniformity from sample to sample. As a result almost each species presents a unique matrix problem. The following discussion of metal analysis of biological samples is therefore devoted to atomic absorption spectrophotometric techniques emphasizing the isolation of the metal under consideration by either separation (arsenic) or matrix correction (other metals).

A. Preparation of Biological Samples for Trace Metal Analysis

Wet ashing of biological samples provides an adequate way of breaking down organic matter with little chance of loss of metals due to volatilization, except mercury, which is considered elsewhere. Middleton and Stuckey (1954) have

described a wet ashing procedure using nitric acid at low temperature. A modification of this technique is presently widely used. After drying at 80° C samples are digested using fuming nitric acid directly. Care must be exercised when using fuming nitric to avoid ignition upon heating. If this become troublesome it is best to add the fuming nitric and allow the sample to stand thirty minutes before applying heat. If the sample should ignite at any point, it should be extinguished with redistilled water immediately to avoid volatilization of trace metals. Also, hotplate temperatures must be below 300°C to avoid trace metal volatilization.

Samples should be covered with a watch glass during the digestion to avoid the rapid evaporation of the nitric acid. Volatilized fats may deposit a film on the watch glass and may be removed with Kimwipes.

After the residue becomes dry, fuming nitric acid should be added to moisten the residue (approximately 1.0 ml). This process is repeated until a white residue remains. At this point the residue is dissolved with HCl and diluted to volume. This residue may not be completely soluble in HCl due to the presence of metaphosphates. This small amount of insoluble matter may be removed by filtering the sample through Whatman #4 filter paper prior to bringing it to volume.

Vycor beakers are used to avoid possible leaching or adsorption of trace metals. Fused quartz beakers are preferred. Beakers having less than 250 ml capacity should be avoided since the smaller surface area will lengthen digestion time considerably.

Blanks are prepared by heating fuming nitric acid in a clean vycor or quartz beaker. Upon evaporation the residue is dissolved with HCl and brought to volume. Blanks for all trace metals considered here should be below detectability by atomic absorption spectrophotometry.

This wet ashing technique has many advantages over dry ashing and other wet ashing techniques. For example, the loss of trace metals (mercury not included) through volatilization is eliminated since the temperature never exceeds 300° C, and sample digestion time is significantly less and requires very little personal attention.

Procedure

(1) To 0-5 gm of dried sample in a 250 ml vycor beaker, add 5.0 ml of fuming nitric acid and cover with a watch glass.
(2) If there is danger of ignition, allow sample to stand 30 minutes.
(3) Heat on a hotplate at 300° C.
(4) When the residue becomes dry, add 1.0 ml of fuming nitric acid. Repeat as many times as necessary to produce a white residue.
(5) Dissolve the residue with 1:1 HCl. If the residue is not completely soluble, filter through Whatman # 4 and dilute to the desired volume.

B. Arsenic

Colorimetric methods for the determination of arsenic in biological material lack sensitivity and specificity. Portmann and Riley (1964) describe a method involving a co-crystallization with thionalide and the subseqent formation of arsenomolybdenum blue complex after digestion. The method, although being specific and sensitive enough, is terribly laborious and time consuming.

Atomic absorption spectrophotometry has until recently been poor at best for the determination of arsenic. The reason for this is that the major arsenic resonance line lies in the ultraviolet region and is difficult for some older spectrophotometers to resolve. Also, the most commonly used flame in atomic absorption work (air-acetylene) absorbs 65-60% of the transmitted UV light. By using an argon-hydrogen-entrained air flame, absorption is cut to 15-20% of the transmitted light at the 193 mμ resonance

line. However, the low temperature of the argon-hydrogen flame encounters interferences in real samples due to incomplete salt dissociation and molecular absorption. This necessitates either matching the sample matrix very closely or chemical separation.

The generation of arsine gas has the advantage of concentrating and separating the arsenic from the original sample matrix. The arsine is generated by the addition of zinc to an acidic sample solution in a reaction flask. The zinc liberates hydrogen from the acid which reacts with As(III) to liberate arsine (AsH_3). Holak (1969) collected the arsine in a liquid nitrogen cold trap, subsequently warming and sweeping it into an argon-hydrogen flame with nitrogen. Madsen (1971) found that arsine is rapidly absorbed by a dilute silver nitrate solution. The silver nitrate solution can subsequently be introduced into the flame; however, the lower limit of sensitivity of the procedure is approximately 1.0 µg. Dalton (1971) reported that the arsine may be carried directly into the flame by the excess hydrogen generated. For this purpose Fernandez (1971) described a collection balloon attached to the reaction flask to collect the arsine and excess hydrogen that is generated. Reproducibility is difficult to obtain due to loss of elasticity from acid attack and the balloon often develops pinholes.

Improved sensitivity may be obtained by purging the reaction flask with argon as suggested by the Jarrell Ash Corporation (1971). The arsine gas generated is passed through a hydrogen sulfide absorber prior to introduction into the flame. This may be accomplished by passing the gas through cotton impregnated with lead acetate.

Biological samples should be wet ashed at low temperature with fuming nitric acid in order to avoid loss of As(V). The final solution should contain no nitric acid so that As(V) may be reduced to As^{III} with KI and $SnCl_2$. This may be accomplished by heating the samples while blowing CO_2

over the samples until dense white fumes cease. A sample aliquot containing between 0.25 and 1.00 µg of arsenic is diluted to a 25 ml volume with a 20% HCl - 5% H_2SO_4 diluent.

At 5X scale expansion, standards in the range of 0.25 to 1.25 µg arsenic give an average absorption of 15% and 75% of the scale, respectively.

Equipment

Beckman model 495 atomic absorption spectrophotometer equipped with a 10 inch linear recorder or other equivalent equipment. Optimum pressure for the argon and hydrogen are 15 and 3 PSI, respectively.

Special Apparatus

(1) 50 ml pear shaped, two neck flask ℱ 14/20.
(2) Adapter with aerator assembly (Scientific Glass JA 7970).
(3) Adapter, reducing ℱ 14/20 - ℱ 24/40.
(4) Polyethylene drying tube.
(5) Flowmeter.
(6) 3-way stopcock.
(7) 2 ml auto-dispenser.

Reagents

(1) Potassium Iodide 20% W/V solution.
(2) Stannous chloride 20% (W/V) in conc. HCl.
(3) Zinc powder suspension (10 gm zinc dust/20 ml redistilled water).
(4) Lead acetate 10% (W/V) solution.
(5) Diluent 5% H_2SO_4 20% HCL solution.
(6) Arsenic standard 1000 ppm solution - 0.4165 gm Na_2H AS O_4. 7 H_2O/100 ml of diluent. All subsequent dilutions must be made with the diluent.

Apparatus Set-Up

The inlet of the aerator assembly is connected to an auxiliary argon supply with tygon tubing (Figure 4.12). This connection should be intercepted with a flow meter and a 3-way stopcock, respectively. The three way stopcock allows the argon to bypass the system. The outlet

side of the aerator is connected to the drying tube containing cotton impregnated with lead acetate. The tygon tubing leading from the H_2S absorber is connected to the burner by way of a hypodermic needle connected to capillary tubing.

Procedure

(1) After removal of HNO_3 using CO_2, a 1.0 ml aliquot of the sample is diluted to a 25 ml volume with the diluent.

(2) Add 1.0 ml of the KI solution and 0.5 ml of $SnCl_2$, mix and let stand at least 15 minutes.

(3) Place the sample in the reaction flask. Adjust the stopcock so that the auxiliary argon purges the system for 30 seconds. Readjust so that the argon now bypasses the system.

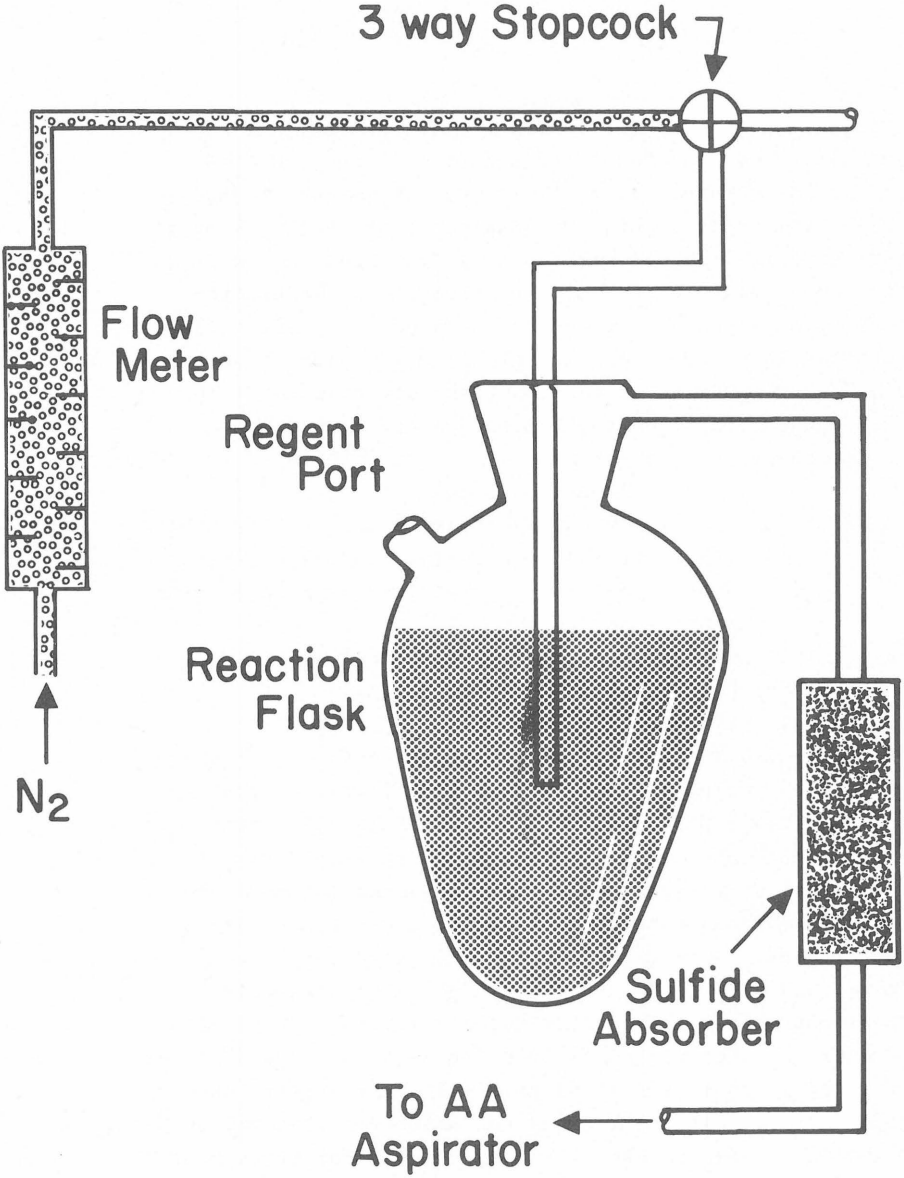
(4) Add 2.0 ml of the Zn suspension with the auto-dispenser and quickly stopper the flask.

(5) When the recorder shows a shift in the absorbance signal, turn the stopcock in order to purge the system with argon.

(6) The absorbance peaks very quickly and returns to the baseline. After the absorbance has peaked, turn the stopcock so the argon will bypass the system.

C. Mercury (Norman Cutshall in collaboration with J. R. Naidu). This procedure is used for the measurement of the total mercury content of tissue samples from marine organisms. The analytical system on which the method has been developed is built around a Perkin-Elmer Model 303 instrument fitted with a 20 cm absorption cell. The 300 cc volume of the closed-cycle gas phase affords a lower limit of detection of 1 ng of mercury. For the procedure described this corresponds to 2 ng/g wet weight concentration in the sample.

The sample is digested with a combination of sulphuric and nitric acids and hydrogen peroxide in order to dissolve the



ARSINE GENERATOR

FIGURE 4.12

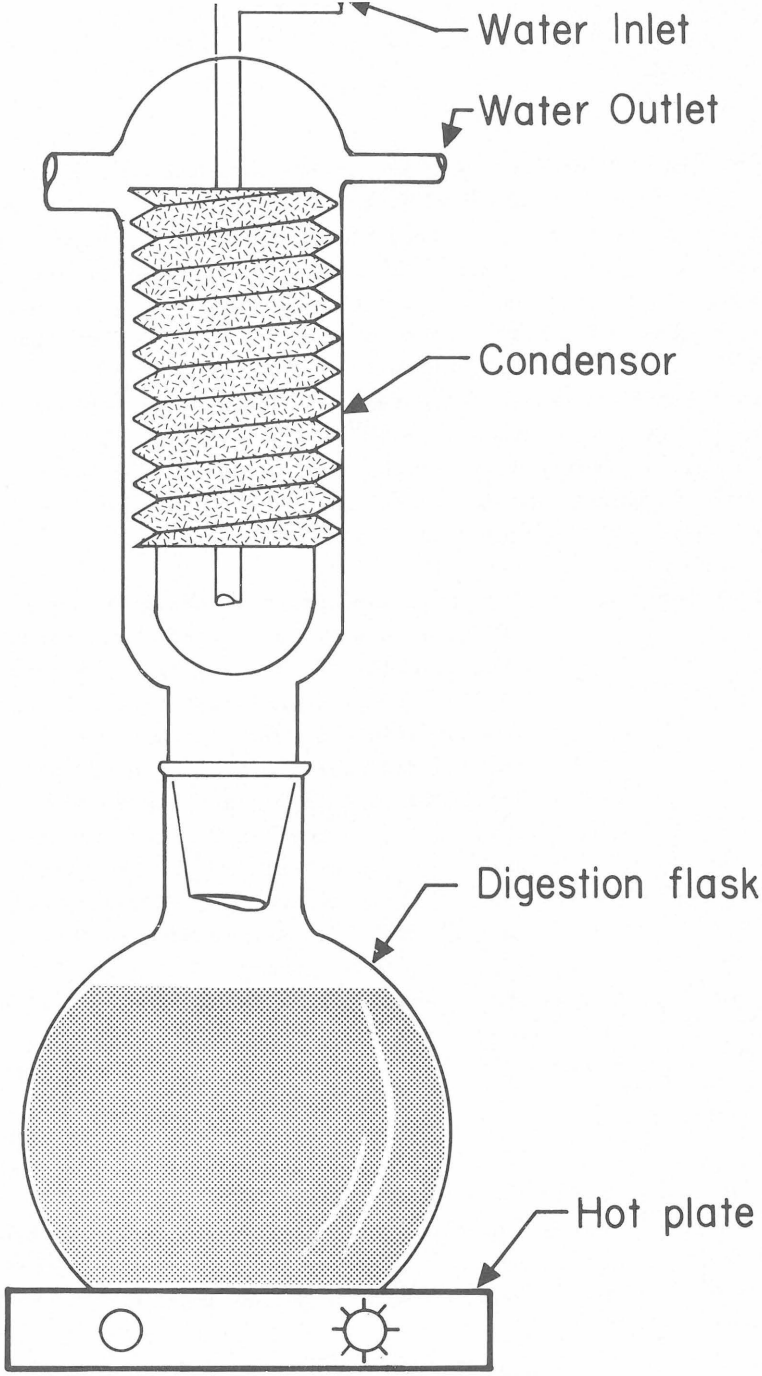
tissue and to convert the mercury to inorganic Hg(II). An aliquot of the resultant solution is reacted with Sn(II) and hydroxylamine to reduce the mercury to Hg(O), and the mercury vapors are swept from the solution by an air stream. The air stream is cycled through a dessicant to remove water vapor and then through the absorption cell positioned in the optical path of a mercury hollow cathode lamp. The quantity of mercury present is determined by comparison of the resultant attenuation of the 253.7 nanometer line to the attenuation caused by standard mercury solutions.

Digestion Step

Apparatus: The digestion is carried out in a flat bottom flask, fitted to a reflux condenser (Friedrich). Heat is applied to the flask from below using a hotplate (Figure 4.13).

Reagents: Concentrated Nitric Acid
Concentrated Sulphuric Acid
Hydrogen Peroxide 30%

Approximately 5 to 10 grams of freshly thawed sample are accurately weighed into the digestion flask. A few boiling beads are added, and the flask is attached to the reflux condenser. (If it is desired to report results on a dry or ash weight basis, another tissue subsample may also be weighed out for determination of dry:wet and ash:wet ratios.) Ten ml of concentrated nitric acid is added and allowed to stand overnight with no applied heat but with the condenser coolant water on. The temperature is then raised to 50°C for an hour. The flask is allowed to cool, and 10 ml of a 1:1 (v/v) mixture of concentrated nitric acid and concentrated sulphuric acid is added. Temperature is raised to 60°C for three hours. Ten ml of 30% hydrogen peroxide is added in 1 ml increments allowing the foaming to subside between additions. Temperature is increased to 80°C for 1 hour, after which the digestion is complete.



MERCURY DIGESTION APPARATUS
(BIOLOGICAL)

FIGURE 4.13

The flask is chilled with an ice bath and the condenser walls rinsed down with 50 ml of deionized distilled water. The chilled solution is filtered through glass wool and diluted to 100 ml in a volumetric flask using deionized distilled water. The digested solution may be kept under refrigeration (5-10°C) for as long as 6 months. The solution should be brought to room temperature just before analysis. Each batch of samples digested includes a reagent blank run as well as a standard run. The blank is 10 ml of deionized distilled water, and the standard is from the working mercury standard. No sample is added to either the blank or standard runs.

Analysis Step

Instrumentation: An atomic absorption spectrophotometer is fitted with an absorption cell as shown schematically in Figure 4.14. A recorder readout is very convenient although not absolutely necessary. The absorption cell should have a path length of approximately 20 cm and a diameter of about 2 cm. Windows are quartz of not more than 0.3 cm thickness. The water vapor trap is loosely packed magnesium perchlorate. The system is connected with 1 cm Tygon tubing.

Reagents: 1 N Sulphuric Acid (Dilute 28 ml of concentrated sulphuric acid to 1 liter.)

Reducing Agent (Dissolve 100 ml of concentrated sulphuric acid, 5 g of sodium chloride, 5.3 g of hydroxylamine hydrochloride and 20 g of stannous chloride in sufficient deionized distilled water to make one liter of solution. Reducing agent should be made fresh daily.)

Mercury Stock Solution (Dissolve about 0.14 g of mercuric chloride in 100 ml of 1 N sulphuric acid. The solution contains a nominal 1 mg/ml of Hg. Solution should be prepared monthly.)

Working Mercury Solution (Working standards are prepared daily by successive dilution of the mercury stock solution in 1 N sulphuric acid to obtain a nominal 100 ng/ml solution. This solution should be calibrated against a suitable standard.

Five ml of the digested solution is added to the reaction flask and sufficient 1 N sulphuric acid added to bring the total volume to 20 ml. Three ml of the reductant solution is added by pipette, and the flask is closed. The flask is gently swirled by hand for one minute and the air pump then started. If a recorder readout is used the absorbance will rise to a maximum within 30 seconds and then fall off to a steady level after about one minute. When the absorbance reaches the steady level the bypass valve is opened and the system exhausted through the mercury trap. When the absorbance reading has returned to zero, the bypass is closed and a volume of the working mercury solution is added to the reaction flask. The volume added is selected to approximate the quantity of mercury found in the analysis run. If the quantity of mercury found is too high or too low for optimim precision, the run is repeated using an appropriate volume of the digested solution. The reagent blank from the digestion step is generally carried through the analysis step with a batch of samples.

Calculations

The wet weight concentration of mercury in the sample is calculated as:

$$C = \frac{(S - B)\ (Hg)_{ref} V}{R \times W \times 100ml},$$

where: S is the sample absorbance, B is the blank absorbance, $(Hg)_{ref}$ is the quantity of mercury added in the reference solution, V is the volume of digested solution added, R is the reference absorbance and W is the weight of sample originally digested.

Note on Reagent Contamination

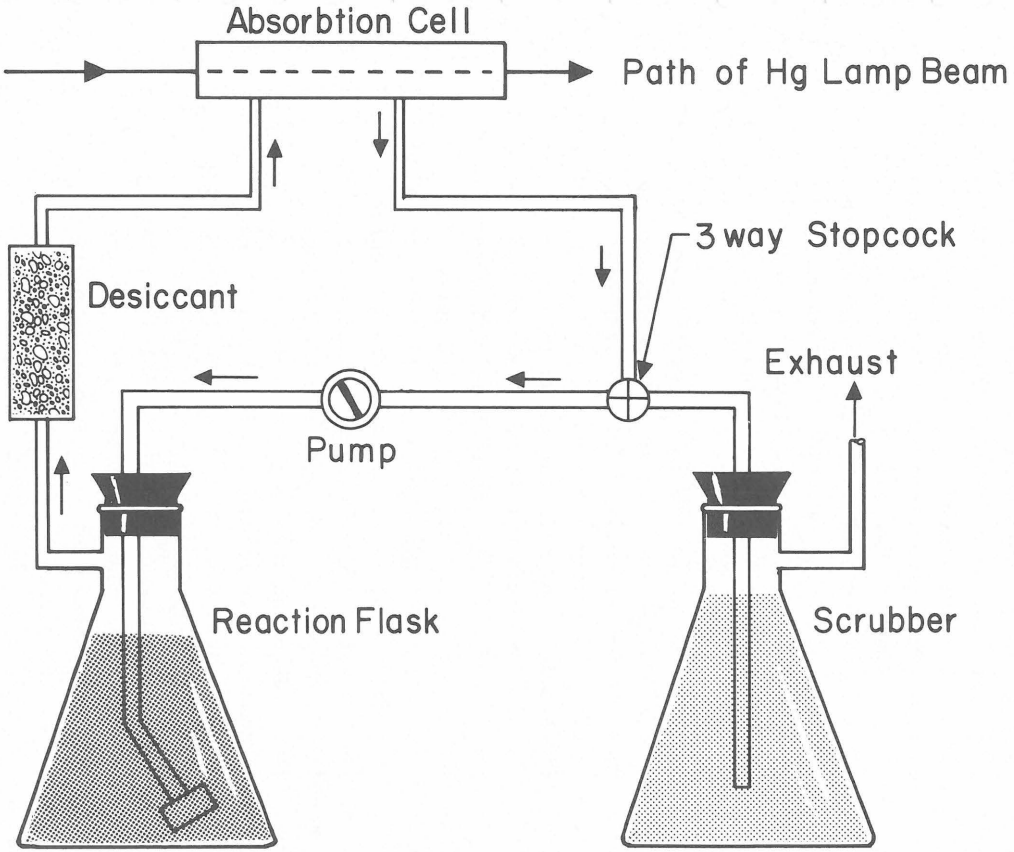
Reagent grade chemicals are used throughout the total analytical scheme. Nevertheless every batch of chemicals including those certified "mercury free," should be run for contamination. The calculation given above makes no allowance for contaminated reducing agent. If the reducing agent gives an absorbance when run directly and cannot be purified, this source of error should be taken into account in the calculations.

Standards and Reference Materials

Mercury standard solutions for calibration of the working standard are available from Fisher Scientific Supply and from Beckman.

Standard solutions containing inorganic and organic mercury are available from the Environmental Protection Agency. Since methyl mercury is much more toxic and has different environmental pathways than inorganic mercury, cases arise when one would like to know the fraction of the total mercury present as methyl mercury.

In most fish and other organisms the bulk of the mercury is apparently present as methyl mercury (Westöo, 1969), so that the procedures outlined for total mercury are in fact measuring within a factor of two the methyl mercury levels. In other organisms or water/sediment systems, much less of the total mercury may be methyl mercury, so techniques which determine specifically methyl mercury are desirable.



APPARATUS FOR THE ESTIMATION OF MERCURY

FIGURE 4.14

To date, essentially all the methyl mercury determinations have been by the gas chromatographic techniques which have been comprehensively reviewed by Sumino (1968) and Fishbein (1970). The biological material is hydrolyzed with hydrochloric acid, for example, the methyl mercury is isolated and purified as methyl mercury chloride and its concentration determined by electron capture gas chromatography. The Swedes have been the leaders in developing and applyint these techniques (Westöo, 1969; Jensen, 1969).

D. Other Metals

For the analysis of other metals in biological samples the initial digestion is the same as that described for arsenic. Once the proper dilution of the sample is made so that the metal concentrations are in the optimum working range for atomic absorption but piror to analysis, the matrix of the sample solution must be established. In the cases of arsenic and mercury, the problem with matrix interferences is corrected essentially by a separation technique. For other metals, separation is time consuming, difficult, and not necessary if proper steps are taken. Atomic absorption spectrophotometry is relatively free of interferences in comparison to other spectral methods. However, interferences do exist, the most serious for heavy metals being molecular absorption. This interference is due to blocking or absorbing of some of the light passing through the flame. Calcium is reported to be the most serious interfering element (Angino and Billings, 1967); however, other specific element interferences in trace metal analysis have been observed and are listed in Table 4.9 for the metals of concern here. Biological samples are particularly susceptible to this type of interference because of the high concentrations of alkali and alkaline earth metals present. Standard addition is not effective in dealing with this type of interference. The most effective means of dealing with the interference is by simply determining the amount of interfering elements

present in the sample. When a trace blank and standards are prepared with the same concentration of interfering elements found in the sample, the interfering signal is subtracted from the total signal.

Table 4.9 Specific Element Interferences

Metal	Calcium	Magnesium	Sodium	Potassium	Iron
Cadmium	X	X	X	X	X
Cobalt	X	X	X	X	X
Copper	X	X	X	X	
Lead	X	X	X	X	
Nickel	X				X
Zinc	X	X	X	X	X

Matching sample and standard matrices of every sample is virtually impossible and unnecessary. At concentrations of the interfering element where its ratio to the concentration of the element of concern is less than 500:1, the interference is almost undetectible. At concentrations exceeding this the interference increases continuously with increasing concentration of the interfering element. It is only important however that sample and standard matrices do not differ by more than about 500 ppm in the concentration of the interfering element for acceptable precision. This therefore allows for grouping of samples with similar matrices.

Of all the metals considered in this section, atomic absorption is inadequate only for the analysis of lead at the concentrations commonly occurring in marine organisms. The sensitivity of the lead analysis and the common interferences encountered with marine samples make atomic absorption of little value for many of the larger organisms such as finfish. Samples containing higher concentrations of lead, such as molluscs, however, can be adequately analyzed by this method.

Sediment Samples

Atomic absorption spectrophotometric techniques are quite adequate for metal analyses at the ranges encountered in organic

rich, clay rich marine and estuarine sediments. Procedures for analysis of marine sediments by atomic absorption spectrophotometry are essentially analogous to those described for biological material using standards made up to duplicate the matrix of the samples. The main difference in the analytical techniques used for these two types of samples is in the digestion procedures. Several different digestion procedures are available which allow the analysis of different fractions of a given sediment. Essentially two digestion techniques are considered in this section. These are: (1) total digestion, when the total concentration of the metal in the sediment sample is desired, and (2) preferential leach, when an estimation of the concentration of authigenic metals and those absorbed on clay particles or associated with carbonate material is desired.

A. Total Digestion of Sediment Samples

Sediment samples may be totally digested by the removal of silica. This may be accomplished with hydrofluoric acid. Upon gentle heating, the silica is volatilized as silicon tetrafluoride. After the complete destruction of organic matter with perchloric and nitric acids, the residue may be brought into solution with 1:1 hydrochloric acid. This analytical procedure follows that described by Hendrick (1968).

Digestion should be carried out in teflon beakers having high acid and heat resistance. The beakers and all glassware used should be cleaned before use by submerging them in hot nitric acid and rinsing them with redistilled water.

Reagents

(1) Hydrofluoric acid (48%)
(2) Nitric acid (conc.)
(3) Perchloric acid (70%)
(4) Hydrochloric acid (conc.)

Procedure

(1) Weigh 0.50 gm sample onto weighing paper.
(2) Cover the bottom of a teflon beaker with redistilled water. Dump the weighed sample into the water.

(3) Add 15 ml of HF and 10 ml conc. HNO_3 to each sample.
(4) Cover with a teflon beaker cover and allow to stand two hours.
(5) Add 2 ml of $HClO_4$ and heat on hot plate at low temperature (setting of 3) until dense fumes of perchloric acid subside.
(6) Allow to cool, and wash the sides of the beaker with a minimum of redistilled water.
(7) Evaporate again to dryness.
(8) Dissolve the residue with 4 ml of hot 1:1 HCl and dilute to a 10.0 ml volume.
(9) Determine matrix and prepare standards accordingly.

B. Preferential Leaching of Trace Metals From Sediments

Chester and Hughes (1967) have investigated several chemical techniques for the separation of partitioned trace metals from pelagic sediments. Among the techniques investigaged were: the use of EDTA (Goldberg and Arrhenius, 1958), 1 M - hydrochloric acid (Arrhenius and Korkish, 1959), dilute acetic acid (Hirst and Nicholls, 1958), and reducing agents (Arrhenius and Korkish, 1959). The effect of EDTA was found to be very slow, while its destruction was found to be difficult. Ray *et al.* (1957) have shown that 1 M - hydrochloric acid will attack the lattice structures of certain clay minerals. The effect of dilute acetic acid on the ferrous and manganese oxide phases of ferro-manganese nodules was determined by these authors as well. Their findings indicate that dilute acetic acid is not sufficient to completely dissolve the iron oxide present in the nodules. Reducing agents were investigated and the use of 1 M hydroxylamine hydrochloride was found to dissolve 50% of the iron oxide present. A combined acid-reducing agent solution was investigated by Chester and Hughes (1967) and found to dissolve 90% of the total iron and 96% of the total manganese from nodules. They also investigated the effect of the combined reagent on the

other major pelagic sediments. Their results indicate that the trace metals leached from clay minerals are compatible with those expected to be found in non-lattice positions. The following is a description of this technique.

Reagents

(1) Acetic acid 35% (v/v).

(2) Hydroxylamine hydrochloride 25% (v/v) solution

(3) Mixed acid-reducing reagent. (Mix 150 ml of 25% hydroxylamine hydrochloride and 350 ml of 35% acetic acid.)

(4) Nitric acid (conc.)

Procedure

(1) Place 0.1 gm of powdered air dried sediment in a 50 ml Erlenmeyer flash.

(2) Add 10 ml of the mixed reagent and stopper the flask. (Do not use a rubber stopper).

(3) Shake the flask on a mechanical shaker for four hours.

*(4) Filter the solution through a Whatman #40 filter paper, and wash the residue and filter paper with a small volume of redistilled water.

(5) Add 1 ml of conc. HNO_3 to the filtrate.

(6) Evaporate the solution at low temperature to near dryness until strong fumes of HNO_3 are evolved.

(7) Cool the filtrate and dilute to 5.0 ml in a volumetric flask with redistilled water.

* Samples should be carried through this step in one working day.

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Heated Furnace Atomic Absorption Technique (K. K. Bertine)

This technique has been successfully applied to the determination of Zn and Cd in waters of low salt content. The exact analytical technique varies with the different makes of heated furnace instruments.

In general, the procedure consists of accurately pipetting 5 - 50 µl aliquots of the water to be analyzed directly into a graphite or tantalum container. The water sample is then dried, ashed, and volatilized at different temperatures for different lengths of time depending upon the element. The following procedure applies only for the Perkin Elmer graphite furnace.

For Zn, a 10-50 µl aliquot of sample or standard is pipetted with an Eppendorf pipette into the graphite rod. The sample is dried for 40 seconds at 100°C, ashed for 40 seconds at 500°C, and volatilized at 2000°C for 15 seconds. The resultant peak is measured with an x-y recorder. Each sample is repeated 6 times and an average taken. Standards covering the sample range are prepared by diluting a 1000 ppm Zn solution with quartz distilled water. A blank is run on the distilled water. The method is practical to levels of <0.01µg Zn/l.

For Cd, a 50 µl aliquot is pipetted into the graphite rod. The sample is dried at 100°C for 40 seconds, ashed at 300°C for 40 seconds, and volatilized at 1500°C for 15 seconds. The resultant peak is measured with an x-y recorder. Each sample is repeated 6 times and an average taken. Standards covering the sample range are prepared by diluting a 1000 ppm Cd solution with quartz distilled water. A blank is run on the distilled water. This method is practical to about 0.01 microgram Cd/l.

%

ANALYTICAL TECHNIQUES FOR SELECTED ORGANIC COMPOUNDS

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Introduction

The organic pollutants discussed in this chapter are limited to polychlorinated biphenyls, DDT and its degradation products and hydrocarbons. The procedures for collection and analyses of marine samples for chlorinated hydrocarbons and petroleum have many common features. Both procedures are subject to extraneous organic contamination of the sample during collection or processing. For this reason, the use of plastic, rubber, paper products, and fiberglass should be avoided. Containers and implements of glass, metal, or porcelain which have been rinsed with pure solvents can be used. During collection, constant attention must be paid to the ship's discharges of bilge, sewage, and refuse so that any sample suspected of contact can be discarded. The safest method of preservation of samples for analysis is freezing at -20°C. Samples collected with these precautions can be used for either petroleum or chlorinated hydrocarbon analyses, or crude extracts of the samples may be subdivided for both analyses if a grease-free homogenizer is used.

Only persons thoroughly familiar with the details of these analyses should be involved in the sample collection. It is highly recommended that the chemist who will perform the analyses also take part in the collecting. Since so many difficulties and hazards are associated with the analytical procedures described in this chapter, only competent professional scientists should be assigned to the work. These are not routine procedures and should never be assigned to non-technical or unskilled

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personnel. It is essential that the analyst prove his ability to produce comparable data by participating in an interlaboratory intercalibration.

Finally, since we strongly recommend that only professional scientists perform the collection and analytical procedures, many otherwise unproductive hours at sea can be profitably used if a small laboratory is installed on the collecting ship. Most of the procedures described in this chapter can be completed at sea.

Recommended Intercalibration Materials

A. Reference Material

Commercial cod liver oil spiked with 10 ppm of Aroclor 1254 and 1 ppm each of p,p' - DDT and p,p'-DDE and 0.5 ppm of p,p'-DDD. This known standard should be sealed under nitrogen in glass ampules (1-5 ml).

B. Research Material

(1) Shark or tuna muscle homogenized with 5 times its weight of anhydrous sodium sulfate. This standard should be packed in wide mouth glass jars with wide screw caps fitted with Teflon liners. About 25 g of the homogenate is sufficient for replicate analyses.

(2) Standard reference material for PCB's, DDT and petroleum to be supplied in a 5 ml glass ampules sealed under nitrogen.

A solution in triolein or a similar pure lipid, liquid at room termperature (18°C) of:

(a) 10 isomers of PCB from trichlorobiphenyl to octachlorobiphenyl each to be present in a concentration between 1 and 100 ppb

(b) p,p' DDT, p,p' DDE and p,p' DDD each to be at a concentration between 1 and 10 ppb

(c) n-alkanes: n-C15, n-C17, n-C18, n-C23, n-C28, n-C33 (1-10 ppm)

(d) branched alkanes: 6 isoalkanes and anteiso-alkanes between C16 and C25 (1-5 ppm)
isoprenoids: Faresane, pristane, phytane and squalane (1-10 ppm)

(e) alkenes: 6 of the α- olefins between C15 and C25 (1-10 ppm) squalene: (10-20 ppm)

(f) cycloalkanes: decalin, a dimethylcyclohexane, camphane, a dimethyl decalin, perhydrophenanthrene, (1-10 ppm)

(g) aromatics: a dimethylbenzene, a dimethyl naphthalene, phenanthrene, biphenyl, tetrahydronaphthalene (1-10 ppm).

Petroleum

We will mainly suggest methods of analysis for petroleum contamination in the ocean based on the experience of ourselves and colleagues. But first we will briefly discuss the nature of petroleum and the principles underlying the analytical methods to be suggested. We assume that anyone attempting to use these methods has a basic knowledge of organic chemistry, analytical chemistry, biochemistry and that he understands the problems associated with analyzing trace quantities of any chemicals. He must also understand problems associated with sampling at sea.

Crude oils and most of their distillation products are extremely complex mixutres of organic chemicals of which hydrocarbons are the most numerous and abundant. Hydrocarbons comprise more than 75% of most crude oils and fuel oils. The review by Bestougeff (1) and the description of many crude oil analyses by Smith (2) are recommended as good introductions to the composition of petroleum. The extreme complexity of crude oils has prevented a complete analysis of all the compounds present. This complexity is thought to result from molecular scrambling which occurs as petroleum is formed (3-5). Whitehead and Breger (6) in 1962 listed over 200 hydrocarbons, 90 sulfur containing organic compounds, 33 nitrogen containing organic compounds. In addition, there are porphyrins, trace metals, and residues in many crude oils called asphaltenes.

The bulk of the reports of petroleum contamination in ocean samples have dealt with hydrocarbons, but bulk oil spill samples and oil samples believed to be from spills have also been analyzed by using trace metal analyses (7), gas

chromatography with flame photometric detector for sulfur compounds (8), infrared analysis (9), and U.V.-fluorescent spectrometry (10) which can detect compounds other than aromatic hydrocarbons that absorb in the U.V. region and/or fluoresce. We are not aware that analyses for compounds other than hydrocarbons have been used for estimating the petroleum contamination of marine organisms aside from the U.V.-fluorescent type (11). It is our opinion that there is not sufficent data and experience concerning the analyses of non-hydrocarbon components of petroleum to incorporate any of the methods into a monitoring program at this time, thus we will limit further discussions to hydrocarbons.

Hydrocarbons

Analyses of marine samples for petroleum hydrocarbons are complicated for two principal reasons. First, organisms synthesize hydrocarbons. Thus the analyst must distinguish between recently biosynthesized or native hydrocarbons and petroleum hydrocarbons in order to estimate petroleum contamination. Second, hydrocarbons in petroleum - crude oils, fuel oils, lubricating oils - are very complex mixtures. Thus the analyst is faced with the problem of identifying a complex mixture of contaminant compounds in the presence of a few, similar, native compounds. The distinction can only be made by comparing hydrocarbons native to marine organisms with petroleum hydrocarbons to see what the distinguishing characteristics of each group are.

Petroleum Hydrocarbons

Crude oils and most crude oil products, e.g. fuel oils, contain a series of n-alkanes with chain lengths of carbons atoms numbering between 1 and 60. The ratio of abundance of odd chain lengths to even chain lengths is approximately 1.0. A series of branched alkanes are also present including isoprenoid alkanes such as pristane, farnesane, and phytane, naphthenes (cyclic alkanes with or without side chains), aromatic hydrocarbons (from alkyl substituted benzenes and

naphthalenes to polynuclear aromatic structures), and naphthenoaromatics (naphthenes joined with aromatic ring systems). Lubricating oils ususally do not contain many n-alkanes because of dewaxing procedures during production which remove the relatively high melting waxes (higher molecular weight n-alkanes). Alkenes (olefins) are not present in crude oils except in a few cases where they have been reported in trace amounts (1). However, they are formed in some refining processes and are present in refined products such as gasoline.

Bestougeff(1) points out that in each homologous series of hydrocarbons types, e.g. n-alkanes, naphthenes, aromatics, there exists families of homologs or isomeric groups (n-alkanes have only one). The result is a complex mixture containing a continuous succession of hydrocarbons over a wide boiling range. This point will be important to the discussion below.

Recently Biosynthesized or Native Hydrocarbons

The hydrocarbons in marine organisms can come directly from the ocean; either picked up from the water directly through permeable surfaces like gills or obtained along with food through absorption from the gut (12,13). Hydrocarbons are more stable than most biochemicals, so it would not be unexpected to find some derived from land sources in marine organisms. Organisms can also make their own hydrocarbons, either *de novo* or from precursor compounds obtained with their food. An example of the latter is the synthesis of pristane from phytol (14).

All of these categories of hydrocarbons occur naturally in marine organisms; they are all of recent biosynthetic origin; they may all be called native to those organisms. We will not distinguish in the following discussion among these various origins but will simply distinguish between the native compounds and those from petroleum sources coming into the ocean mainly as a result of human activities.

The last comprehensive review of hydrocarbons native to organisms was that of Gerarde and Gerarde (15). Clark (16) reviewed the literature on saturated hydrocarbons (mostly non-cyclics) in 1966.

N-alkanes

Both land and marine organisms synthesize n-alkanes. Odd carbon chain lengths predominate, although even carbon chain lengths are present. In many instances one or two predominate over all others. In marine phytoplankton, the n-alkanes with 15, 17, 19 and 21 carbon chain lengths are most abundant (16, 17) whereas in marsh grasses and Sargassum 21-29 odd carbon chain lengths predominate. Some bacteria have been found to contain equal amounts of even carbon and odd carbon chain lengths in the 25-32 chain length region (18). Marine sponges and corals have also been shown to have little if any odd carbon chain length preference for the range of carbon chain lengths between 25 and 34 (19).

Branched Alkanes

Branched alkanes including pristane, an isoprenoid hydrocarbon, have been found in organisms. In some fish pristane is the most abundant alkane present (13). Phytane has not been detected in copepods from the Gulf of Maine nor in Basking shark liver (13).

Alkenes (Olefins)

Alkenes are often a major percentage of the hydrocarbons found in organisms- particularly marine organisms. Squalene is a major constituent of basking shark liver oil and cod liver oil. Isoprenoid 19 and 20 carbon chain length mono, di, and triolefins are present in copepods and some fish (20). Polyolefins such as the 21 carbon hexaolefin- hexeicosahexaene- are predominant hydrocarbons in several marine planktonic and benthic algae (21, 22). Carotenes are polyolefins and are found in considerable quantities in many organisms.

Alicyclic Hydrocarbons

Hydrocarbons containing one to three non-aromatic rings are present in several herbs and other land plants. Most are classified as terpenes because of their biosynthetic origin from isoprene. Several alicyclic saturated hydrocarbons have also been reported.

Aromatic Hydrocarbons

There are several reports of aromatic hydrocarbons as reviewed by Gerarde and Gerarde (15). These have been found in land plants and spices from them.

General

In general it can be said that the analyses of marine and land organisms to date have shown that many different types of hydrocarbons are produced by organisms. However, an analysis of an individual species does not reveal more than a few of each given type of hydrocarbon, e.g. alkanes, alkenes, and many times only one type of hydrocarbon has been detected. The only exceptions to this generalization might be the bacteria, yeasts, and actinomycetes. There is a need for analysis of more of such organisms from the sea, cultured under conditions which would eliminate contamination by petroleum hydrocarbons.

We also wish to point out the fact that a limited number of species (land and marine) from only a few geographic locations have been analyzed for their native hydrocarbons. The stage in the life cycle of the organism at the time of analysis should also be considered, since this can influence the hydrocarbon distribution. Finally, many of the past investigators have limited their analytical techniques in order to search for only one or two classes of hydrocarbons - usually alkanes and alkenes - and would not have detected other classes, if present, or have not reported their presence.

Distinguishing Between Native Hydrocarbons and Petroleum Hydrocarbons

A comparison of the hydrocarbons native to organisms and the hydrocarbons in petroleum shows the following characteristics which would be of use in distinguishing petroleum contamination. Some of the characteristics will not apply for all organisms.

(1) Petroleum contains a much more complex mixture of hydrocarbons with a much wider boiling range than has been reported for native hydrocarbons in any one species of organism.

(2) Petroleum contains a mixture of many aromatic hydrocarbons in comparison with the few aromatic hydrocarbons known to be synthesized by organisms.

(3) Petroleum contains phytane, an isoprenoid hydrocarbon not believed to be present in most biological samples from situations free of petroleum contamination.

(4) Many petroleums contain a homologous series of n-alkanes with a wide boiling range and no predominance of odd carbon chains over even carbon chains.

The best method for detecting petroleum contamination is to have analyses available from specimens free of contamination with which comparisons can be made. Unfortunately, there is no place in the ocean from which organisms can be collected which we can be certain is free from contamination. Even organisms grown in culture may be contaminated by petroleum absorbed from minute amounts dissolved in the culture water unless special precautions are taken.

Methods of Analysis

Analysis of materials for hydrocarbons requires the extraction of all or a portion of lipids including hydrocarbons. The hydrocarbons are then separated from the lipids and analyzed using the criteria just described to distinguish between petroleum derived hydrocarbons and native hydrocarbons.

Solvents and Reagents

All solvents should be redistilled. All reagents should be extracted by Soxhlet extraction using redistilled solvents - a methanol:benzene (1:1) mixture can be used for 24 hours, or if the reagents are stable they may be ignited at 500°C for 12 hours. Aqueous solutions should be prepared from cleaned reagents and distilled water and then extracted several times with redistilled pentane or hexane in a separatory funnel.

Blanks or Controls

It is essential that the entire extraction and analytical procedure in use be carried out periodically in its entirety without a sample to determine if hydrocarbons from the solvents and reagents are interfering with the analyses of samples.

Marine Organisms

Of the several methods for lipid extraction, we are most familiar with two: (1) Soxhlet extraction with methanol or methanol:benzene(1:1) and (2) homogenization in pentane or hexane with anhydrous Na_2SO_4 in a Virtis homogenizer.

Soxhlet Extraction

Single thickness Whatman extraction thimbles are extracted in the Soxhlet apparatus with methanol:benzene (1:1) for 24 hours. The thimbles are cut below the top of the siphon of the Soxhlet extractor. The sample is then weighed into the thimble and extracted for 24-48 hours with methanol or with methanol: benzene (1:1). If the solvent in contact with the sample after the 24-48 hours is colorless this is a good indication that the extraction is completed. However, we recommend a check for completeness of extraction for each type of sample, i.e. different species or different tissues and organs, by extracting the sample with a fresh pot of solvent for an additional 24 hours and working this extract up separately.

The methanol:benzene extraction is preferred when a sample is suspected of being particularly rich in lipids, e.g. a fish liver. In either case the extract is allowed to cool and then is extracted three times with 1/3 its volume of pentane. The combined pentane phases are then washed with 1/3 volume of distilled water saturated with NaCl, dried over anhydrous Na_2SO_4 and evaporated to dryness at reduced pressure; usually 200-100 mm of mercury is a good setting for ambient temperature and below. Careful monitoring of a manometer connected to the vacuum line allows the analyst to see the sudden drop in pressure when all of the pentane or pentane-benzene is removed. The flask is quickly removed from the evaporator to prevent losses of lower boiling hydrocarbons.

The residue is taken up in a small volume of pentane and an aliquot weighed to provide an estimate of the extracted lipids.

Virtis Homogenization

We have employed this procedure on occasion to extract lipids from organisms, especially when the lipid extract was split with Dr. George Harvey (see section on PCB's) for analysis of samples for chlorinated hydrocarbons. A sample weight of 20-50 g wet weight is placed in a Virtis Homogenizer bowl with 4 to 5 times its weight of anhydrous Na_2SO_4 and extracted with 100 ml of pentane or hexane for 2 minutes at high speed. The pentane is carefully poured off through a 2-4 cm/diameter, 8-10 cm long column of anhydrous Na_2SO_4 into a round bottom flask or Kudernaner Danish evaporator flask. The extraction is repeated twice with fresh solvent. Finally the Na_2SO_4 in the Virtis bowl is poured onto the column, and the column is rinsed with 50-100 ml of pentane. The extract is concentrated by rotary evaporation at reduced pressure or in the Danish Kuerdner evaporator fitted with a ball joint condensor and placed in a hot water or steam bath. Aliquots of the lipid residue or concentrate are then weighed to determine the amount of lipid extracted.

We do not recommend the use of ordinary blendors, since contamination of the sample with bearing lubricant may occur, unless the blender blades are disassembled and cleaned.

Saponification

Most lipid extracts contain esters of fatty acids -- waxes and glycerides. These often interfere with the isolation of pure fractions of alkenes and aromatic hydrocarbons. Saponification of the lipid extract prior to isolation of the hydrocarbons breaks the esters into fatty acid salts and alcohols which are easily removed so as not to interfere with later hydrocarbon measurements.

The lipid extract free of pentane is refluxed for 2 hours in a solution of 1 part 0.5 N KOH in methanol, 1 part benzene, and 0.5 parts water. After cooling, the solution is transferred to a separatory funnel, and the non-saponifiable lipids are partitioned into pentane. The pentane-benzene extract is washed with water saturated with NaCl and then the solvents are

removed by evaporation at reduced pressure on a rotary evaporator. It is essential that the water can be present in the amount given above to prevent transesterification of esters to methyl esters which would interfere with the isolation of the hydrocarbons. Infrared spectra of the non-saponificable lipid extract contains no absorption band due to the ester function if the saponification is complete.

Small samples of lipid extract can be saponified in a 12-15 ml centrifuge tube with Teflon lined screw cap. The lipid extract is heated for 20-30 minutes in a boiling water bath with 5-6 ml of the saponification solution described above. The tube is flushed with N_2 prior to heating and shaken periodically during the reaction. After cooling the non-saponifiable lipids are partitioned into pentane by adding pentane to the tube, shaking, and centrifuging. The pentane layer is drawn off and the procedure repeated twice. The pentane extracts are combined and evaporated to yield a non-saponifiable lipid residue.

Notes on Emulsion Problems

If problems with emulsions occur during any of the above procedures they can be minimized by slowly adding enough water saturated with NaCl to break the emulsions. Very stable emulsions may require centrifuging or passage through a column of previously ignited sand.

Chromotographic Separation of Hydrocarbons from Other Lipids

Column chromatography and thin layer chromatography have both been used successfully in separating hydrocarbons from other lipids. The analyst adjusts the operating procedures to his or her particular needs. Activities of column and thin layer absorbents can vary from batch to batch, and careful monitoring is required to be sure that the desired separations are accomplished. We recommend that mixtures of standard hydrocarbons representing each class of compounds be prepared and used to adjust operating procedures to obtain the desired separations.

Column Chromatography

We have routinely employed a column of Al_2O_3 packed over SiO_2 in a ratio of 1:1 or 2:3, respectively. The absorbent to lipid ratio should be at least 50:1. Both the Al_2O_3 and SiO_2 should be Soxhlet extracted for 24 hours with methanol:benzene (1:1), activated for 24 hours at 250°C and 120°C respectively, and then deactivated with 6% water and 5% water, respectively. Deactivation should be by thorough mixing and standing for 24 hours. We use Harshaw Chemical Company AL 0102P Al_2O_3 and Davison Chemical Grade 922(200-325 mesh) SiO_2. The column should have a length to diameter ratio of about 20:1. The SiO_2 is packed first using either a dry packing technique or wet packing technique followed by wet packing the Al_2O_3 when the SiO_2 is well packed at the bottom of the column.

The sample of non-saponifiable lipids is charged to the column in a very small volume of pentane and washed into the column with two small volume rinses of pentane. The method of elution is a matter of choice as discussed above. We have employed several variations of 1-4 column volumes (after collecting void volume) of pentane followed by a 1 column volume each of varying percentages of benzene in pentane and benzene alone. With increasing numbers of column volumes and increasing percentages of benzene, more polar hydrocarbons and eventually some pigments (including carotenes which are hydrocarbons) are eluted from the column.

It may be desirable to collect several classes of hydrocarbons in a combined eluate and then re-chromatograph the hydrocarbons on an activated column such as SiO_2 to obtain a better separation of the classes of hydrocarbons (for example see Blumer and Sass ref 27).

The eluates of the column are usually monitored by gas chromatography and U.V. spectrometry or U.V.-fluorescent spectrometry to determine which eluate fraction contains the various classes of hydrocarbons. The weight of hydrocarbons in each eluate can be obtained by weighing an aliquot of concentrated eluate on a Cahn Electrobalance. If a large

amount of hydrocarbon is present--greater than 1 mg--a less sensitive balance may be used. With proper care to avoid contamination the sample may be recovered from the weighing procedure.

Thin Layer Chromatography

Thin layer chromatography has been employed by Quinn. [(Farrington *et al.* (24)] to separate hydrocarbons from other lipids. Silica Gel G plates 0.37 mm think were predeveloped in chloroform and air dried. The lipid or non-saponifiable lipid extract was applied to the plate and the plate developed in petroleum ether: ethyl ether: acetic acid (95:5:1). The plates were also spotted with appropriate standards of hydrocarbons prior to development. The standard spots and sample hydrocarbon band(s) are visualized by brief exposure to iodine vapors; the appropriate bands scraped from the plates; the hydrocarbons eluted from the silica gel; and the hydrocarbon extract is concentrated by rotary evaporation at reduced pressure.

Ehrardt (25) has used column chromatography to isolate hydrocarbons from most other lipids in an oyster extract. Thin-layer chromatography was then employed to separate or partially separate 5 fractions of hydrocarbons. Silica Gel F-254 on aluminum 20 x 20 cm (E. Merck, A. G.) was activated at 228° C for 15 minutes, cooled, and the sample was immediately applied. The plates were developed in isooctane and the bands located by viewing the plate under U.V. light.

Infrared Spectrometry

This technique shows little promise in detecting small quantities of petroleum hydrocarbons in the presence of native hydrocarbons. The absorption frequencies for native hydrocarbons overlap or coincide with the adsorption frequencies of petroleum hydrocarbons with only a few possible exceptions for aromatic hydrocarbons in the low wavelength region. This could be applicable only in the situation where there were no

native aromatic hydrocarbons. We prefer other more sensitive techniques for distinguishing between native and petroleum hydrocarbons.

U. V. Spectrometry and U. V. Fluorescent Spectrometry

These methods of analysis indicate the presence of aromatic hydrocarbons (U.V.) and polynuclear aromatic hydrocarbons (U.V.-Fluorescence). We have used U. V. analysis to monitor our column eluates to detect aromatic hydrocarbons. Absolute quantities can only be guestimated since the extinction coefficients are difficult to determine if the components are unknown. There are also limitations in using this technique. No indication of the presence of alkanes or non-conjugated alkenes is given. There is little indication of the complexity of the mixture or the boiling range, and there is the possibility of interference with the absorption spectra of the aromatic hydrocarbons by conjugated alkenes. This limitation can be overcome by hydrogenating the hydrocarbons with Adams Catalyst (PtO_2) in hydrogen at atmospheric pressure (22). The olefins will be hydrogenated but not the aromatic hydrocarbons. The U. V. spectra then can be ascribed to the aromatic hydrocarbons alone.

The use of U.V. fluorescent analysis in detecting polynuclear aromatic hydrocarbons has been described (10, 11).

Gas Chromatography

We have found this technique to be most useful in screening samples for petroleum contamination. Temperature programmed gas chromatographic analysis from 80-290°C at 6° per minute on 2-3.5% Apiezon L on Chromosorb W(80/100) 6.0-10 ft with flow rate adjusted for maximum number of plates yields suitable chromatograms. A recent study by Zafiriou *et al.* (26) has shown OV-101 SCOT (support coated open tubular) columns, 50 ft x 0.02 in (Perkin Elmer Corporation) with 25,000 effective plates to be a very good column for petroleum hydrocarbon analyses. Other non-polar phases such as SE-30 and polar phases such as FFAP have also been used for analyzing hydrocarbons, although we find them less suitable for analyzing petroleum hydrocarbons.

Hydrocarbon fractions are dissolved in CS_2 for gas chromatographic analysis since CS_2 gives a minimum solvent peak.

For the purposes of a more reliable identification of individual peaks as particular hydrocarbons we have employed gas chromatography before and after hydrogenation on both the Apiezon L column and a 12% FFAP on Chromosorb W (80/100) 10 ft 1/8 in ss column using 100-260°C at 4-6°/minute temperature programming. The hydrocarbon by co-injection of authentic standards or by determining Kovats retentionindices peaks in the gas chromatograms are tentatively identified.

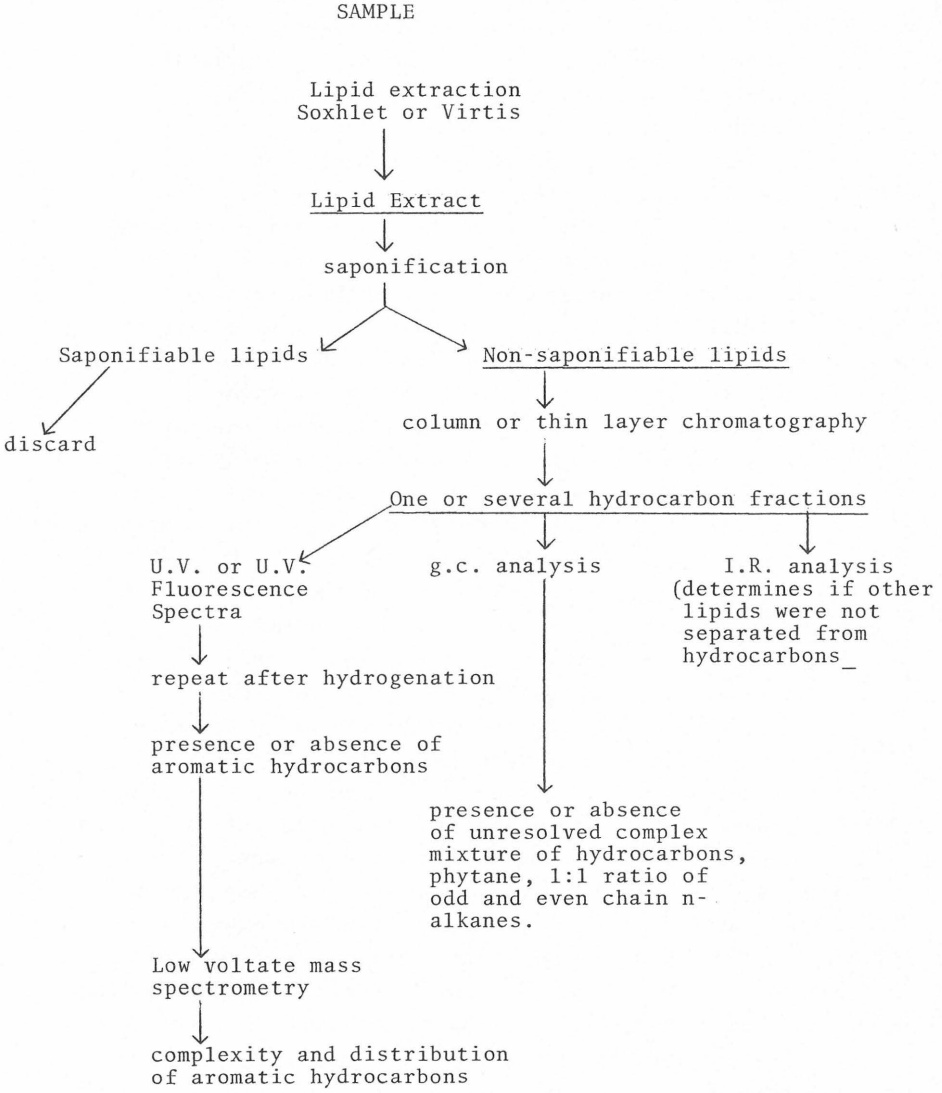
Gas chromatography can also be used to quantitate the hydrocarbons in a sample. The detector response of the known fraction of a sample injected to the gas chromatograph is compared to the response of a known amount of standard injected to the gas chromatograph under identical conditions. Alternatively, a spike of known amount of an n-alkane may be added to the sample at the time of extraction and used as an internal standard.

Mass Spectrometry and Gas Chromatography-Mass Spectrometry

Hydrocarbons isolated from shellfish and sediments contaminated by petroleum have been analyzed by g.c. mass spectrometry and by mass spectrometry alone (25, 27). This is particularly effective for detecting petroleum contamination when low voltage mass spectrometry is used to analyze the aromatic fractions isolated from a sample. The mass spectra obtained show nominal mass numbers of fragments of aromatic hydrocarbons, e.g. in the case of aromatic hydrocarbons from a sample contaminated with petroleum, fragments of the series of alkylated aromatic hydrocarbons of a distribution and complexity similar to that found in petroleum itself are present in the mass spectra (25, 27).

Recommended Procedure for Determining Petroleum Contamination

The following is an outline of the procedure we recommend for analysis of hydrocarbons in marine organisms to detect petroleum contamination.

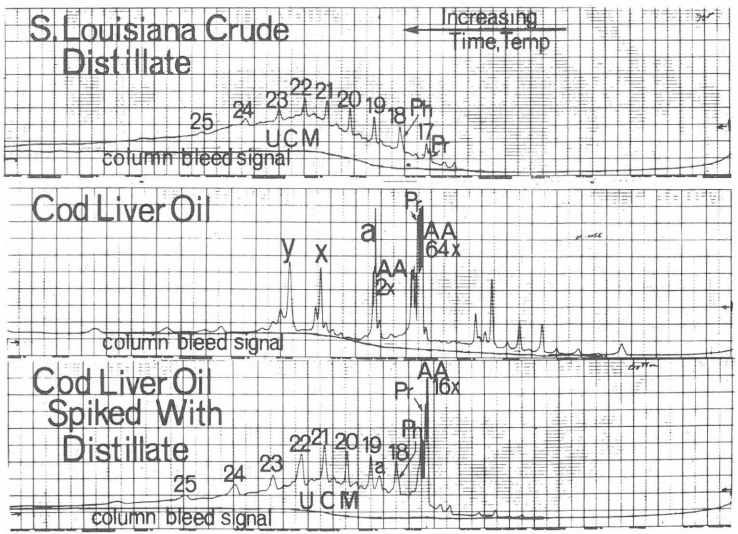

SAMPLE
Lipid extraction
Soxhlet or Virtis
Lipid Extract
saponification
Saponifiable lipids
discard
Non-saponifiable lipids
column or thin layer chromatography
One or several hydrocarbon fractions
U.V. or U.V. Fluorescence Spectra
repeat after hydrogenation
presence or absence of aromatic hydrocarbons
Low voltate mass spectrometry
complexity and distribution of aromatic hydrocarbons
g.c. analysis
presence or absence of unresolved complex mixture of hydrocarbons, phytane, 1:1 ratio of odd and even chain n-alkanes.
I.R. analysis (determines if other lipids were not separated from hydrocarbons

This procedure provides information in each of the categories of characteristics for petroleum hydrocarbons listed on page 159 and 160. Gas chromatography provides information about the complexity and boiling point distribution. It also detects the presence or absence of phytane and the 1:1 ratio of even and odd carbon n-alkanes. In addition gas chromatography provides the information about native hydrocarbons and their absolute and relative abundance. Figure 5.1 shows a gas chromatogram of a distillate cut from a crude oil, a gas chromatogram of hydrocarbons isolated from a cod liver which contained no detectable quantities of petroleum, and of the hydrocarbons isolated from the same cod liver extract spiked with the distillate cut of crude oil. The 1:1 correspondence of the even and odd carbon n-alkanes can be seen in both the upper and lower gas chromatograms, as can phytane from the petroleum. The unresolved complex mixture signal is due to the elution from the column of many superimposed and overlapping peaks which result from the very complex nature and wide boiling range of petroleum hydrocarbons. This feature is present in both the upper and lower gas chromatograms. The simplicity of the distribution of hydrocarbons found in most marine organisms is shown by the middle gas chromatogram.

We caution here that the characteristic 1:1 ratio of odd and even n-alkanes may be misleading if found since several bacteria, as previously mentioned, have been reported to have native hydrocarbons of such a distribution. The ratio may also be hard to detect in the presence of native n-alkanes such as are found in marsh plants, sponges, and corals. The absence of n-alkanes with such a ratio may mean that the sample does not contain petroleum contamination. This can be confirmed by the absence of other characteristics for petroleum hydrocarbons, or it could mean that the petroleum contaminants have been bacterially altered prior to or after incorporation into the organism. N-alkanes are much more susceptible to bacterial attack than are other petroleum hydrocarbons (5).

The procedure outlined on page 168 also provides information about the presence or absence of aromatic hydrocarbons and

Figure 5.1 Gas Chromatograms of Hydrocarbons in Pentane Eluate of Column Chromatography.



AA. Automatic attenuation of detector signal Pr- pristane; Ph-phytane; numbers -n-alkanes, a-phytadienes; x, y unknown hydrocarbons; UCM- Unresolved complex mixture. Conditions of analysis were those described for Farrington (24). GC column was 3% Apiezon L. Detector sensitivity set at 10^{-11} x 16.

their complexity and molecular distribution. Thus we believe that careful application of the suggested procedures will provide information about the presence or absence of petroleum contamination in marine organisms and also provide an estimate of the severity of contamination.

There are of course several modifications of the methods of analysis which can be employed to suit each occasion. The method is also limited to petroleum hydrocarbons boiling between n-C_{12} and n-C_{34} for the present. We have not discussed the use of gel permeation chromatography nor high pressure liquid chromatography, because we believe that these methods have not been sufficiently field tested to recommend them for a monitoring program.

Sea Water Analysis

Petroleum and its by-products persist in the ocean for long enough times for detectable concentrations to accumulate. Our knowledge of the pathways and rates of transport of petroleum pollutants through organisms to their fates of sedimentary deposition or biological oxidation depends on accurate measurements of concentrations of dissolved and particulate petroleum in seawater. A simple method which permits isolation, purification, and measurement of the materials follows.

Freshly collected seawater is extracted in a continuous liquid-liquid extractor using pentane or hexane as the solvent. If only dissolved hydrocarbons are of interest the sample should first be filtered through a glass-filter (pre-cleaned). The glass-fiber may be taken through the procedure already described for marine organisms to identify the particulate hydrocarbons present. Twenty liters of sea water is an adequate sample if the total normal paraffin concentration is in the 0.1 to 1.0 microgram per liter range.

The liquid-liquid extractor consists of two 12 l round-bottom flasks, a condenser, and a 250 ml distillation flask. The samples are best extracted overnight so that purification can be done each morning. Hexane does not extract the total

lipids of seawater. However, in control experiments the apparatus described gave recovery of 95% for n-paraffins with more than 20 carbon atoms. The significant losses of smaller paraffins could be reduced by using the pentane as solvent and lowering condenser temperatures. After an overnight extraction the sample of hexane, about 100 ml, was removed from the distillation pot and purified according to the scheme already described. Since the amounts of materials are very small micro-glassware should be used. For example an eyedropper serves as a suitable silica-gel chromatography column.

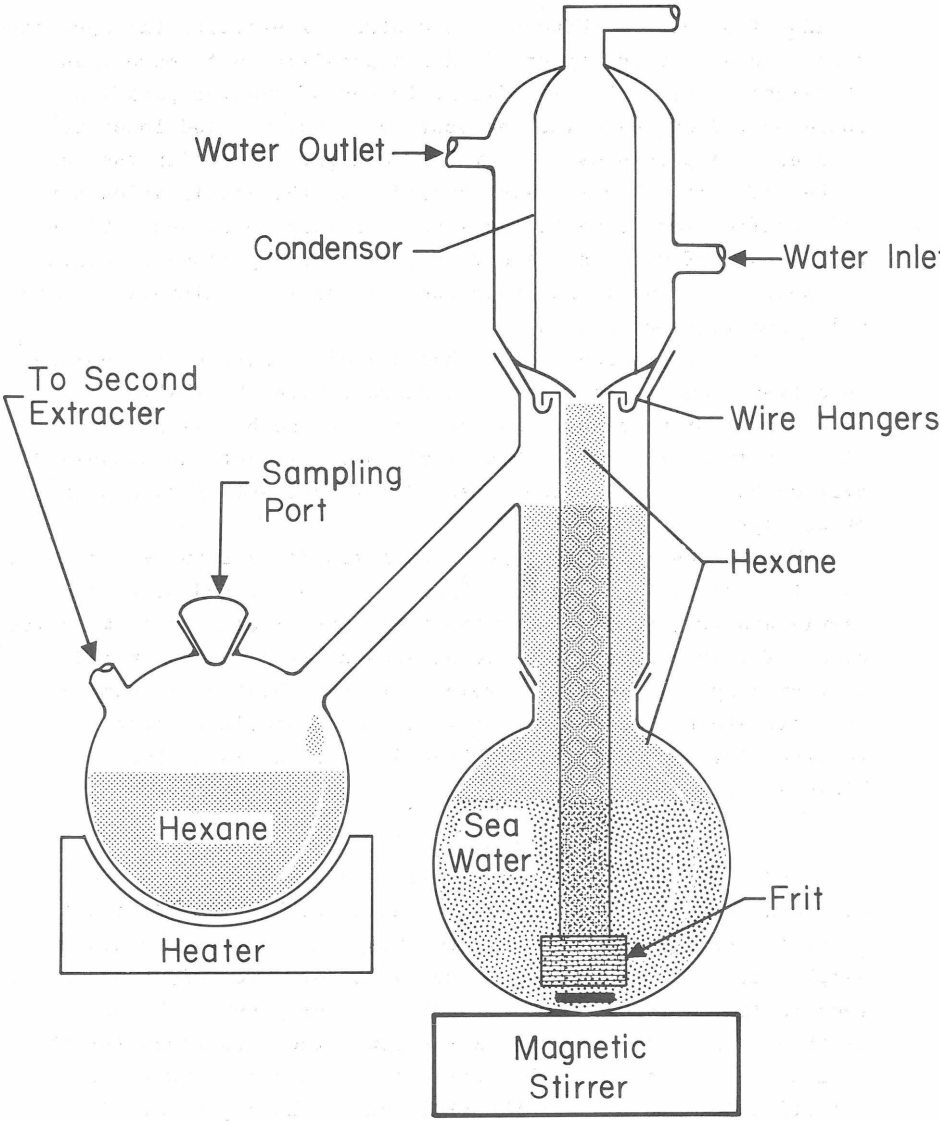
A simpler procedure which has the disadvantage of requiring more time from the analyst is to extract water by shaking it with hexane or pentane. Shaking water thoroughly with 10% of its volume of pentane three times will extract the dissolved hydrocarbons which are then treated like extracts obtained in other ways.

The success of this type of analysis depends on two factors; one, the use of a carefully collected and preserved seawater sample and two, care to keep the experimental blank contamination down. The apparatus, once clean, should not be disassembled between samples. In case samples with very high petroleum contents are processed, the extractor used should be then reserved for such samples or cleaned by firing in a glass-blowers furnace.

Sediment Analysis

Sediment samples can be analyzed by using the same procedure as described for marine organisms with a few additional steps in the procedure. It is advantageous to stir sediment samples containing >40% H_2O in methanol for a few minutes and then to filter through pre-extracted filter paper. The sediment is then extracted using the extraction method described for the analysis of organisms. The extraction should be interrupted from time to time to stir the sediment in the cup to avoid "channeling" in the sediment sample.

The methanol wash, which removes the bulk of the water, is combined with the extract and analyzed as described for organism



CONTINUOUS LIQUID-LIQUID EXTRACTOR FOR PETROLEUM

FIGURE 5.2

extracts with the addition of a sulfur removal step. An active copper column can be used to remove elemental sulfur from lipid extracts or the hydrocarbon isolates. Elemental sulfur is carried through the analytical procedure with hydrocarbons and interferes with their analysis.

Copper may be prepared by precipitation from a mixutre of a powdered zinc slurry and copper sulfate solution. An alternate method is to activate commercially prepared powdered copper with 2N HCL and rinse thoroughly with distilled water and methanol. The copper from either procedure may be packed in a column through which the lipid extract or hydrocarbon -S isolate is slowly perculated, or it may be shaken with the extract or hydrocarbon-S isolate in a centrifuge tube. The formation of black CuS indicates that S was present.

Bulk Oil Samples and Tarballs

The analyses of bulk oil samples and barballs have been described in detail by several authors (8-11, 26, 27, 29, 30). A method of correlating oil spill samples with sources has been recently described (26). Tar samples have also been analyzed by these methods. Gas chromatography of bulk samples can be accomplished by dissolving the sample in CS_2, by removing the insoluble residue and water by centrifuging, and drawing off the CS_2 (26). Alternatively the bulk sample may be directly introduced to the gas chromatograph using a technique described below.

"A small amount of oil (or tar ball) (0.2 - 0.8 mg) is placed in one end of a glass tube (2.5 mm od, 1.5 mm id) which fits into the injection part of the gas chromatograph. The nut holding the septum is removed, the glass tube is inserted quickly into the injector with the oil-containing end first, and the septum is replaced immediately. The volatile components of the oil are permitted to distill into the cold column (air- or dry ice-cooling) from the hot injector (200°C). After 5 minutes the glass tube is withdrawn, and the oven temperature programmed at a rate of 6°/min from the 50-290° (Apiezon L) or from 50-275° (FFAP)." (29).

Glassware used for bulk oil samples and tar samples should be thoroughly cleaned and blanks analyzed to ensure that no oil contaminants remain before using the glassware for low level contamination analyses. If possible it is desirable to keep the size of the sample introduced to the gas chromatograph approximately the same as the size for low level analyses to avoid "gunking up" the column.

Analyses of bulk oil samples and tarballs more detailed than gas chromatographic correlation type analyses can be accomplished by using the same procedures as outlined for organism extracts. Many bulk oil samples and tar samples will need to be de-asphalted prior to column chromatography. This can be accomplished by shaking the sample with hot hexane and centrifuging to remove the aspahlt residue.

Reference

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Analysis for Polychlorobiphenyls and DDT Compounds in Marine Samples

Introduction

While many methods (1-5) are available for the analyses of chlorinated hydrocarbons, the analytical methods for polychlorobiphenyls (PCB) and DDT (6) presented in this report are somewhat specific for the marine environment because marine samples reflect the following differences from continental or agricultural samples:

(a) generally lower concentrations,i.e., 10-300 ppb in biota and 1-50 ppt in water.

(b) no serious interference from other chlorinated hydrocarbons such as chlordane, dieldrin, etc.

(c) PCB concentrations are generally higher than DDT.

Organisms residing in the mixed layer generally reveal PCB chromatograms that are practically superimposable on standard commercial mixtures (AROCLORS). The same is generally true of surface water chromatograms. Some abyssal and mesopelagic water and organisms show significant alteration of the PCB chromatographic fingerprint in their extracts, and thus mass spectral confirmation is desirable.

These methods for analyzing organisms have been checked and verified against those from many laboratories in the U. S., Canada, and abroad in several intercalibration exercises. These procedures gave excellent recoveries (80% or better) based on these intercalibrations. It is strongly recommended that before any laboratory sets out on a monitoring program it assures itself of the validity of its techniques by participating in an international or interlaboratory calibration. Finally, these methods have been applied to a wide variety of marine samples (7-13).

This report was written for experienced chemists who may not be particularly familiar with the problems associated with PCB or DDT analyzed or work at sea.

Procedure

A. Sampling and Preservation

All samples should be taken with non-plastic samplers which should be rinsed with chlorinated hydrocarbon-free solvents

(e.g., ethanol) before use. They may be stored in mason glass jars fitted with aluminum foil-lined caps; jars, foil, and caps should be pre-washed with chlorinated hydrocarbon-free ethanol. Samples are deep-frozen (-20°F); if a freezer is not available, they may be preserved in 5% aqueous formalin (the latter solution should be analyzed also.

B. Reagents

(1) Acetone-technical Grade
(2) Acetone-technical grade distilled in glass.
(3) Acetonitrile (Pesticide grade)
(4) Benzene (Pesticide grade)
(5) Distilled water: doubly distilled water, redistilled from potassium permanganate
(6) Ethanol
(7) Ethanolic KOH (2% w/v)
(8) Ether, diethyl (A. R.)
(9) Florisil (Floridin Co.)
(10) Hexane (Pesticide grade)
(11) Petroleum ether, bp. 30-60 (Pesticide grade)
(12) Silical gel 60-200 Mesh.
(13) Sodium chloride (A. R.)
(14) Sodium sulfate (A. R.)
(15) Standards: Hexane solutions containing 3 to 4 ng/ul of either Aroclor 1242, 1254 or 1260 are convenient.

C. Decontamination of Equipment and Reagents

The factors limiting the sensitivity and accuracy of analyses are due primarily to background contamination introduced by reagents and equipment. Hence, efforts should be made to keep the "noise level" of the resultant chromatograms at a minimum.

All equipment except blenders are rinsed with a primary wash of technical grade acetone. Then, the labware is soaked overnight in tap water saturated with a laboratory detergent. It is scrubbed and rinsed thoroughly with hot tap water and distilled water, then placed in a 300+°C

oven to heat at least 10 hours. The equipment is removed just before use and rinsed with acetone and petroleum ether.

Other non-combustibles used in the extraction, such as sodium sulfate and sodium chloride, glass wool, and aluminum foil are also heated at 300+°C overnight. The blenders are washed and rinsed with the solvents, but are necessarily exempted from the heat treatment.

Sharkskin filter paper, when used to separate the extract from tissues, has been found to be a serious source of contamination. Therefore, a few sheets from every box are extracted by petroleum ether and checked by gas chromatography (G. C.) for interference by electron capturing chemicals. Badly contaminated boxes are discarded, acceptable sheets are kept under petroleum ether in a capped glass jar.

Florisil is heated overnight at 130°C and rinsed with 100 ml of petroleum ether prior to analysis.

Immediately before extraction and cleanup, all equipment and reagents are rinsed with ca. 50 ml of petroleum ether. This washing is concentrated to ca. 5 ml and analyzed by G. C. The chromatogram should not exhibit interferring peaks greater than 0.1 ppb of DDT or its metabolites, or rinsing is repeated until a satisfactory chromatogram is obtained. Equipment and reagent blanks should always accompany analyses of samples.

D. General Extraction Procedure

Sample (50 g) is homogenized which is followed by blending with anhydrous sodium sulfate (100 g) and then petroleum ether (150 ml). The blend is carefully decanted through a 8 cm Buchner funnel fitted with sharkskin filter paper into a 500 ml filter flask. The blending and filtering steps are repeated twice using 2 x 100 ml portions of petroleum ether.

The combined filtrates are dried over anhydrous sodium sulfate and transferred with 3 x 5 ml rinses of petroleum

ether to a Kuderna-Danish Evaporator. The volume of extract is concentrated to ca 20 ml and transferred with rinses to a tarred beaker. The contents of the beaker are evaporated to near dryness over a steam bath and under a stream of N_2. The weight of the residue (lipid) is recorded and used below. (If the lipid weight exceeds 3 g, use only 3g).

The lipid is placed in a 125 ml separatory funnel; petroleum ether (12 ml) is used to wash the beaker and added to the lipid. The petroleum ether-lipid solution is extracted 4 times with acetonitrile (4 x 30 ml) which was previously saturated with petroleum ether.

The acetonitrile extract is treated with a saturated sodium chloride solution (40 ml) and distilled water (650 ml). This aqueous acetonitrile solution is extracted twice with petroleum ether (2 x 100 ml). The combined petroleum ether extracts are washed twice with triply distilled water (2 x 100 ml) and then dried over anhydrous sodium sulfate.

The extracts are then transferred with 3 x 5 ml petroleum ether rinses to a Kuderna-Danish concentrator and concentrated to 5-10 ml. The solution is now ready for florisil treatment.

E. Florisil Separation

Preheated and standardized[14] Florisil is placed in a chromatographic 22 mm id glass column, followed by anhydrous sodium sulfate (1 cm thick) and petroleum ether (100 ml). The solvent is drained off until it barely covers the top of the packing.

The solution of petroleum ether extracts from earlier procedures is introduced onto the column. The solvent is eluted until the meniscus just covers the top of the packing. The column is eluted with a 200 ml mixture of diethyl ether (6%) and petroleum ether (94%) at ca. 5 ml/min. The eluate which contains DDT and PCB is received in a Kuderna-Danish Concentrator and concentrated to about 5 ml. The solution is now ready for GC analysis.

F. Alternate Procedure

If possible, several whole fish should be homogenized and sub-sampled. Whole livers must be homogenized before sampling. Generally, a 10 - 20 g is sufficient. The tissue is mixed with four times its weight of sodium sulfate and extracted with three fresh 100 ml portions of hexane in a homogenizer for 2 min. The Virtis homogenizer proved satisfactory. The extracts are decanted and poured through a 1 inch column of sodium sulfate into a Kuderna-Danish concentrator. The concentrator is fitted with a 3 ball Snyder column and immersed in steam of a hot water bath. The extract is concentrated to 10 ml. One ml is removed and allowed to evaporate in a tared dish to determine the % lipid. An aliquot of the remaining 9 ml of extract, equivalent to 1 g or less of lipid is applied to the top of a 13 x 2 cm column of Florisil packed with hexane. The PCB and DDT are eluted with 200 ml of 6% ethyl ether in hexane at 2 ml/min. The eluate is concentrated as before to 1 - 3 ml and transferred to a silica gel column (see below).

G. Silica Gel Separation of PCBs and DDT

(Snyder-Reinert Procedure (15))

The silica gel (4 g) is heated to 200°C overnight. It is cooled and packed with hexane in a glass wool plugged chromatographic column (1 x 20 cm), previously 1/3 filled with hexane. (The column is tapped gently to remove adhering air bubbles). Hexane is drained off and a concentrated solution (ca 1 ml) of sample is placed on the column. The column is eluted by hexane (38 ml) which is used to remove interfering PCBs, followed by benzene (36 ml) which contains DDT. The hexane and benzene solutions are concentrated for GC analysis as before.

H. Gas Chromatography Analysis

Approximately 5 microliters of cleaned-up extract is injected into a GC equipped with an electron capture

detector. The following parameters are merely representative of a typcial analysis for PCBs.

injector T = 220
column T = 200
detector T = 250
flow rate = 40 ml/min of 5% argon - methane
column - 2m x 2mm glass or stainless steel packed with 8%QF-1 and 2% OV-17 (1:1) on Anakrom 100 - 120 mesh.

Under these conditions the PCBs are eluted in 30 min. Quantification: The heights of 6 or more of the matching peaks in the sample and the closest fitting commercial mixture (Aroclors, Kanechlor, Clophen, etc) eluting after DDE are summed and converted into weights by simple arithmetic proportions. This is illustrated by the following example from actual chromatogram of a seawater extract (Figure 5.3). The sum of heights of the 6 peaks checked on the Aroclor 1254 chromatogram is 23 cm and corresponds to 11.2 ng injected. The sum of the same peak heights in the sample is 6.2 cm. Thus:

(1) $\frac{11.2 \text{ ng}}{23 \text{ cm}} = \frac{X \text{ ng}}{6.2 \text{ cm}}$

$X = 3 \text{ ng}$

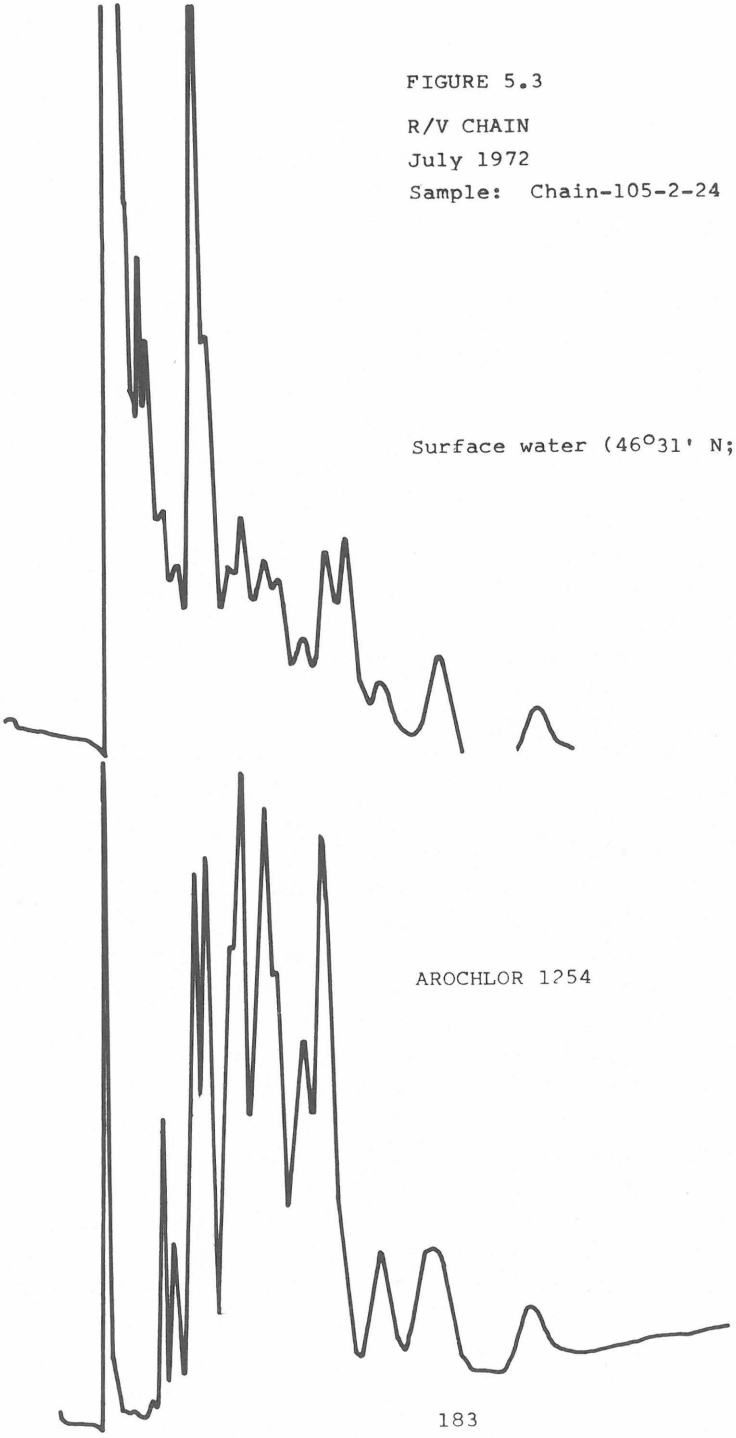
(2) $3 \text{ ng} \times \frac{540}{5.4}$ (aliquot) = 250 ng

(3) $\frac{250 \text{ ng}}{20 \text{ kg}} = 12.5 \text{ ng/kg} = 12.5 \text{ ppt}$

Occasionally, extracts of higher organisms (cod, tuna, sharks etc) or deep benthos reveal such an altered PCB fingerprint that less than 6 peaks match. Quantification by gc alone is unwise and mass spectra should be obtained (4, 16).

FIGURE 5.3

R/V CHAIN
July 1972
Sample: Chain-105-2-24



DDT is also quantified by the above procedures using the appropriate standards. Other columns may be used in confirming DDT or PCB, e.g., Figures 5.4 and 5.5.

I. Confirmation of DDT by Alkaline Hydrolysis (FDA's method (1)).

A concentrated soltuion (1-5 ml) of the sample containing DDT is placed in an Erlenmeyer flask (125 ml) and carefully evaporated to near dryness. 20 ml of ethanolic potassium hydroxide solution (2%) was added to the flask and the contents boiled under reflux for 1/2 hour. The contents are then transferred with 3 x 10 ml rinses of petroleum ether to a 125 ml separatory funnel. The combined ethanol residue--petroleum ether solutions are washed four times with distilled water. The aqueous washings are discarded and the petroleum ether layer is dried over anhydrous sodium sulfate. The petroleum ether extract is concentrated to the original volume and is now ready for GC analysis.

J. Comments on Shipboard Analyses

All of the above-described extractions and analyses have been performed at sea with no unusual difficulties. The work should be conducted in a room that is well ventilated and remote from the main activities of the ship. A portable laboratory unit is ideal. Hexane vapors can be conducted away from the concentrators by means of an oilless vacuum pump attached to a glass funnel fitted to the top of the Snyder column and vented out a port hole. If steam is not available the Kuderna-Danish apparatus can be replaced with an electric heating mantle fitted to a round-bottom flask. No problems have been encountered with the gas chromatograph due to the ship's motion or electrical noise. This is not true for flame ionization detectors. A major advantage of having a complete laboratory at sea is that sources of extraneous contamination can be located and eliminated before any samples are ruined or, sampling programs can be altered to avoid certain types of contamination.

FIGURE 5.4

Tracor MT-220 Gas Chromatograph

Mixture		RRR	RT	Peak
Aldrin	0.016 ng/µl	1.00	3.85	1
o,p-DDE	0.02	1.50	5.72	2
p,p'DDE	0.02	1.87	7.08	3
p,p'DDD	0.02	2.42	9.20	4
p,p'DDT	0.04	3.17	11.95	5

(RRR = Relative retention ratio)
(RT = Retention time, minutes)

G.C. Conditions

Input Atten.	10
Output Atten.	64
Det. Temp.	275°
Col. Temp.	225°
Volume	5 µl
Voltage	38

5% D. C. Chromasorb W (80-100 mesh)

60 cc./min. N_2 carrier gas

^{63}Ni Electron capture detector

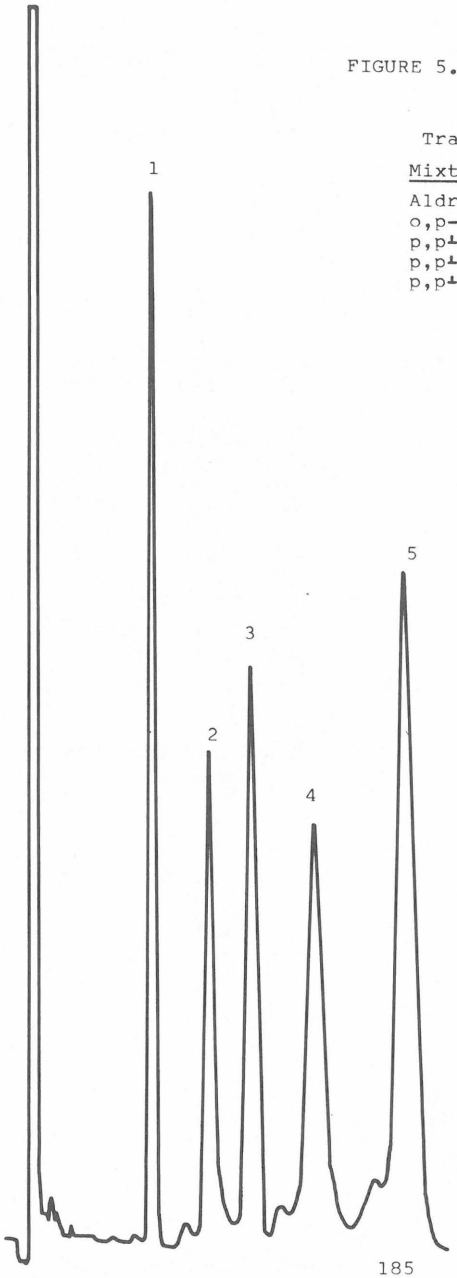


FIGURE 5.5

Tracor MT-220 Gas Chromatograph

	RRR	RT	PEAKS
(Aldrin	1.00	3.85	not included
Arochlor 1254	0.83	3.27	1
0.2 ng/µl.	0.94	3.53	2
	1.31	4.95	3
	1.56	5.89	4
	1.83	6.91	5
	1.96	7.38	6
	2.37	8.95	7
	2.79	10.52	8
	3.31	12.48	9

(RRR = Relative retention ratio)
(RT = Retention time, minutes)

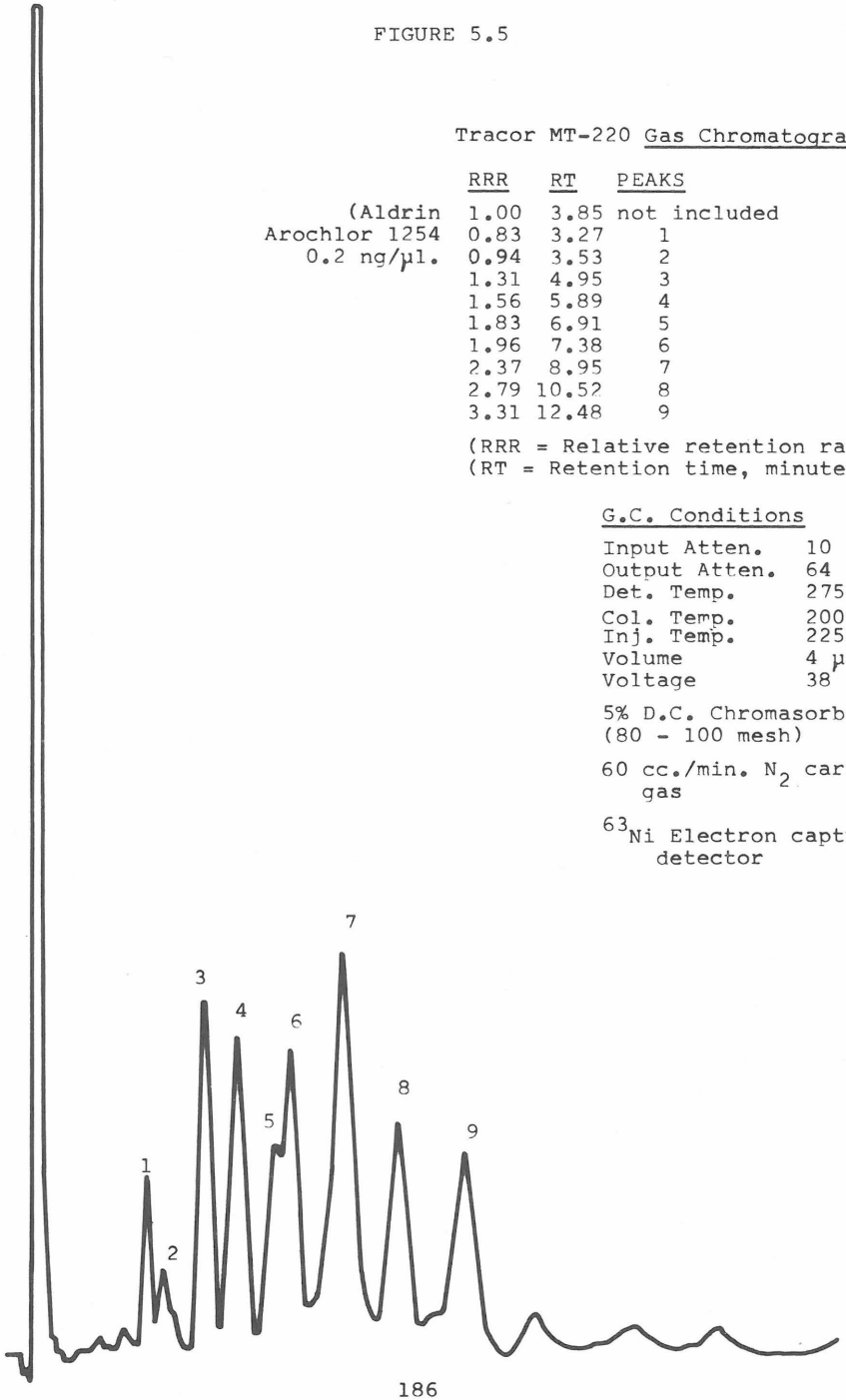
G.C. Conditions

Input Atten.	10
Output Atten.	64
Det. Temp.	275°
Col. Temp.	200°
Inj. Temp.	225°
Volume	4 µl
Voltage	38

5% D.C. Chromasorb W (80 - 100 mesh)

60 cc./min. N_2 carrier gas

^{63}Ni Electron capture detector



K. Special Precautions for Plankton Sampling

Plankton should be washed down the net into the cod end with freshly dipped seawater and allowed to drip free of all water before freezing or extraction. Generally, 10 g is sufficient for an analysis. Plankton nets are quite prone to catching tar balls, plastic detritus, paint chips and oil droplets. Thus, before analysis the sample must be carefully scrutinized and possibly discarded. New and used nets (nylon or silk) can have as much as 13 mg/m^2 PCB adsorbed on them (11). Some of this can be transferred to the plankton while towing. Frequent washing of the net with alcohol is recommended. The above mentioned precautions are most important in coastal areas, harbors, etc. and are usually not necessary in the open sea.

L. Shipboard Collecting Precautions

Before any samples are collected, the ship's oils, greases, paints, and air conditioning supply should be analyzed for sources of PCB. Depending upon the results certain types of sampling may be impossible or have to be greatly altered. All contact of the samples with plastic (except Teflon), paper, cardboard, and rubber should be avoided. Porcelain trays can be used to receive the organisms from the net for sorting. Nets should be stored in large washtubs, be frequently machine washed, and be rinsed with reagent grade alcohol. Surface water is collected by hand dipping a stainless steel bucket from the bow of a ship underway or from the leeside of a ship on station. Water from depth should be taken in a 20 to 40 liter stainless steel sampler. However, a Teflon lined sampler has been used without serious problems. Water must be analyzed as soon as possible after sampling. Organisms should be frozen in glass or prewashed aluminum foil immediately after collection and identification.

M. Extraction of Seawater

Two considerations govern the extraction method for seawater: (a) at least 20 liters should be extracted for reliable results, and (b) the decision must be made on

whether or not to filter. The quantity of water required makes solvent extraction cumbersome and time consuming. The ship's seawater system should never be used since there is usually a considerable amount of PVC tubing in the line. Thus, the most convenient method would be an *in situ* pumping system with simultaneous adsorption onto a solid matrix. Amerlite XAD-2 resin has been used with success (see below), whereas others have used polyurethane foam. Otherwise, 20 liters of seawater can be extracted (2 liters at a time) in a separatory funnel with 200 ml of 6% ether-hexane.

In very polluted or sedimented coastal areas prefiltration is essential before analysis. In other locations one must decide what is desired from the analysis, whether significant particulate matter will be extracted by the procedure and what size particles will be filtered. The solid matrix adsorption has the advantage that only the dissolved phase will be extracted. The following is a description of the use of Amberlite XAD-2 resin (12).

Twenty to fifty liters of seawater is pumped through a 5 cm glass wool plug into a 50 cc column (brass or glass) filled with XAS-2 resin (length: diameter = 7) at a rate of 250 ml/min. The PCB and DDT are eluted from the column with 4-5 column volumes of hot acetonitrile. The acetonitrile is diluted 2:1 with seawater which has already passed through the resin. The PCB and DDT are extracted with 3 x 50 ml portions of hexane. The hexane is washed with 25 ml of water, dried over sodium sulfate, and concentrated. Usually, no further cleanup is necessary before glc analysis.

Concluding Remarks

We have tried to outline procedures, techniques, and precautions for analyses of PCB and DDT in the marine environment that have actually worked well in our laboratories. Other workers in the field could, no doubt, recommend many other variations, refinements, alternatives, and more sophisticated

workups. In the bibliography we have included references to the more elaborate workups for difficult samples.

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(6) DDT residues include DDT and its metabolites DDE and DDD: the major isomers analyzed are the p, p'-isomers; - p, p'-DDT, 1, 1, 1-trichlore-2, 2-bis (p-chlorophenyl) ethane; p, p'-DDE, 1, 1-dichlore-2, 2-bis(p-chlorophenyl) ethylene; p, p'-DDD also know as TDE, 1, 1-dichloro-2, 2-bis(p-chlorophenyl) ethane.

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AIDS TO ANALYSIS

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Calibration Standards--Heavy Metals

The National Bureau of Standards has issued two Standard Reference Materials which are of value for the analysis of marine organisms for their heavy metal content. These materials are orchard leaves and bovine liver which were issued as Standard Reference Materials (SRMs) 1571 and 1577, respectively. A third material, albacore tuna muscle is still undergoing analysis at NBS. Inhomogeneity has been observed for several elements in the tuna, notably Pb, Sr and Ca; other elements, such as Zn, Rb and K are homogeneously distributed throughout the samples. If the homogeneity for the mercury content of this material is satisfactory, it will be issued as an SRM. This decision will be made before 1 January 1973.

Of other standards recommended at the International Decade of Ocean Exploration (IDOE) Baseline Conference (May 24-26, 1972), a lyophilized near shore sediment of fine-grained, non-calcareous, high organic content and a "plankton" standard have been agreed to, in principle, at NBS. Preparation of these standards will not be completed for several years. A low total metal content sample is being proposed at NBS at present. This material is high purity $CaCO_3$ and should be available in 1973.

In the following Tables (6.1 and 6.2) are summaries of all results reported for analyses of orchard leaves and bovine liver SRMs. These Tables may be of use to trace metals analysts.

Collection and Preservation of Water, Sediments, Dry and Wet Fallout, and Organisms for Trace Metal Analysis

Seawater

There have been many references to the contamination of seawater during the sampling process. This may arise from

Table 6.1 Values Reported for NBS-SRM 1571

(Orchard Leaves)*

Element	Range of Results** NBS	Other	Recommended Interim Value	NBS Certified Value
Aluminum	341-428(2)	99-420(14)	400	
Antimony		2.7-3.5(3)	2.9	
Arsenic	11.3-15.9(2)	10-18.3(7)		14±2***
Barium		40-52(5)	45	
Bismuth	0.10-0.14(1)		0.11	
Boron	31-34(1)	23.7-38(13)		33±3
Bromine	9.4-10.5(1)	8.9-9.5(2)	10	
Cadmium	0.09-0.12(2)	<0.1-.45(9)		0.11±0.02
Calcium	2.052-2.125%(2)	1.63-2.41%(15)		2.09±0.03%
Chlorine	680-780(1)	790(1)	750	
Chromium	2.34-2.38(1)	1.8-2.5(2)	2.3	
Cobalt	0.18-0.23(1)	0.1-0.18(5)	0.2	
Copper	10.8-13.7(3)	9.9-20(25)		12±1
Iron	282-319(3)	151-367(21)		300±20
Lanthanum		1.2-2.1(2)		
Lead	41.4-47.9(3)	37-53(12)		45±3
Lithium	14(1)		14	
Magnesium	0.6136-0.6370%(1)	0.40-0.71%(14)		0.62±0.02%
Manganese	88.6-95.0(2)	52-144(2)		91±4
Mercury	0.149-0.170(3)	0.10-0.18(15)		0.155±0.015
Nickel	1.2-1.5(2)			1.3±0.2
Nitrogen	2.723-2.784%(2)	2.50-2.86%(8)		2.76±0.05%
Phosphorous	0.206-0.211%(1)	0.14-0.31%(13)		0.21±0.01%
Potassium	1.444-1.506%(2)	1.11-1.62%(18)		1.47±0.03%
Rubidium	11.0-12.5(2)	10.3-12(3)		12±1
Scandium		0.04-0.2(3)	0.04	
Selenium	0.076-0.091(2)	0.08-0.21(5)		0.08±0.01
Sodium	71-90(3)	40-524(12)		82±6
Strontium		23-45(6)	37	
Uranium	0.026-0.031			0.029±0.005
Zinc	22.8-28.2(3)	18-81(25)		25±3

* Concentrations given in µg/g of dry sample unless otherwise specified.

** Range given is of individual values reported for NBS results and of mean values reported for other laboratories. Number in parentheses is for the total number of groups reporting values.

*** Values reported for arsenic cluster around concentrations of 14 µg/g and 10 µg/g. At the present time a value of 10 µg/g should not be considered erroneous.

Table 6.2 Values Reported for NBS-SRM-1577

(Bovine Liver) *

Element	Range of Results** NBS	Other	Recommended Interim Value	NBS Certified Value
Antimony		<0.1-.014(2)	0.014	
Arsenic	0.050-0.059(1)		0.055	
Cadmium	0.24-0.32(3)	<0.1-0.35(6)		0.27±0.04
Calcium	117-125(1)		123	
Cesium		0.013(1)	0.013	
Chlorine	2533-2656(1)		2610	
Chromium		0.18(1)	0.2	
Cobalt	0.17-0.19	0.15-0.29(3)	0.18	
Copper	181-204(3)	120-202		193±10
Iron	259-299(2)	232-280(6)		270±20
Lead	0.26-0.41(2)	0.33-2.5(3)		0.34±0.08
Magnesium	600-611(1)		605	
Manganese	9.3-11.3(2)	10.4-27.9 (7)		10.3±1.0
Mercury	0.014-0.017(2)	0.006-0.070(7)		0.016±0.002
Molybdenum	3.08-3.34(1)		3.2	
Nitrogen	10.25-11.03%(2)			10.6±0.4%
Potassium	0.93-1.01%(2)	0.756-0.99%(3)		0.97±0.06%
Rubidium	17.5-19.2(2)	16(1)		18.3±1.0
Selenium	1.05-1.17(2)	0.95(1)		1.1±0.1
Silver	0.054-0.081(1)	<0.05-0.11(2)	0.06	
Sodium	0.223-0.267%(2)	0.20-0.267%(3)		0.243±0.011%
Strontium	0.136-0.142(1)		0.14	
Uranium	0.00055-0.00090(1)		0.0008	
Zinc	120-134(3)	78.5-242(10)		130±10

* Concentrations given in μg/g of dry tissue unless otherwise specified.

** Range given is of individual values reported for NBS results and of mean values reported for other laboratories. Number in parentheses is for total number of groups reporting values.

the water sampler used, the hydrowire and/or handling.

A. Seawater Samplers

Most of the non-metallic water samplers today are composed of one or more of the following structural materials: teflon, polyvinyl chloride (PVC), structural nylon or rubber. Robertson (1968) analyzed samples of some of these for their Zn, Fe, Sb, Co, Cr, Sc, Cs, Ag, Cu and Hf contents. Of the above materials, rubber was found to be the most impure. Various examples contained from 0.3 to 4% zinc and sufficient amounts of Cr, Co, Cs, Sb, and Sc so that the amounts actually found in seawater might be altered. PVC contains high amounts of Zn, Fe, Sb and Cu. Teflon and plexiglass tubing were relatively free from trace element impurities.

Which of the commercial samplers available is best will depend upon the particular trace element or elements one desires to measure. In general, those with a minimum of rubber parts will be better. One way of avoiding some of the possible contamination associated with the structural materials is to use samplers which take large water samples (30 liters or greater) so that a large volume of water to surface area is present which would minimize contamination. One other requirement for water samplers is that they must be open during descent so that there is ample flushing of the sampler with the ambient seawater before closing.

B. Hydrowire

Robertson (1968) and Cooper (1958) have indicated that the hydrowire, and in particular the corrosion products from the hydrowire, used to lower water samplers on most ships can contaminate the water samples collected. Of the elements studied by Robertson (1968), Fe, Sb, Co and Cu would be most affected. Cooper (1958) suggested the use of nylon or terylene hydrowires. If such hydrowires be not available, the water samplers should be set as far out from the hydrowire as possible.

C. Handling

All shipboard handling of the water after recovery should be performed in a plastic enclosure away from other activities.

D. Preservation

After collection, the seawater should be stored in 15 liter high density polyethylene containers. Before filling, these containers should be soaked in nitric acid for several hours, then removed and soaked in doubly distilled water for a similar period of time. Prior to insertion of water sample, the containers should be rinsed twice with aliquots of the sample, then filled with the sample. This should all be done in a plastic enclosure as described above.

If the sample has to be filtered, 0.45μ nucleopore filters are preferred. 0.45μ millipore filters contain large amounts of Cr, Zn and lesser amounts of Sb, Co, Sc, Fe and Cs (Robertson, 1968). If millipore filters are used, at least 10 liters should be filtered through the same filter paper to bring the possible level of contamination down to acceptable levels.

Doubly distilled nitric or hydrochloric acid should be added to the water samples to bring them to a pH of 1.8. This prevents the sorption of such elements as Zn, Co and Fe onto the sides of the containers (see for example Spencer *et al.*, 1970). Blanks on the acid chosen should be run for all elements to be determined.

River or Lake Waters

A. Samplers

If deep water samples are desired, the same procedure as for seawater samples should be followed. If surface samples are desired these can be sampled with a high density polyethylene bucket to avoid hydrowire problems.

B. Handling and Preservation

The same procedure should be followed as for seawater samples.

Rain Water

A. Samplers

Rain samples should be collected in high density polyethylene funnel-type collectors draining into 10 liter polyethylene containers. Just before a rain is expected, the collector surface should be thoroughly rinsed with a liter of doubly distilled water. A wet cloth is first used to dislodge dry fallout from the collector. If samples are desired for studies of the variation in the dissolved and particulate contents of elements during the storm, the containers can be changed. Before filling, the containers should be soaked in nitric acid for several hours. After removal of the acid they should then be soaked in doubly distilled water for a similar period of time.

B. Preservation

Doubly distilled nitric or hydrochloric acid should be added to the water samples to bring them to a pH of 1.8. Blank determinations on the acid should be made.

Dry Fallout

A. Samplers

Dry fallout samples are collected in the same type of collectors as are rain waters. During rains, the collector should be covered. After a collection period of 5-7 days, the dry fallout should be scraped from the sides of the funnel with a teflon scraper, then washed into a high density (precleaned as above) polyethylene container with a known volume of doubly distilled water.

B. Preservation

The water is then brought to a pH of 1.8 with doubly distilled nitric or hydrochloric acid.

Sediments

A. Sampling

The primary problem associated with sediment sampling is to recover the top surface of the sediment. Recent work on box cores (Soutar and Isaacs, personal communication) has shown that these devices are best for recovery

of the top sediments. Soutar and Isaacs used a hydraulically slowed box core. The slowed entry allows the core frame to settle before box core penetration and the water trapped above the core to escape at the top of the box.

B. Preservation

Immediately after collection, the core should be quick frozen and then stored at -20°C (Soutar and Isaacs, personal communication). If no varves are present to indicate sampling intervals, the frozen core should be subdivided at one centimeter intervals with depth. The material adjacent to the sawed areas and core liner should be scraped off with a teflon scraper.

Organisms

A. Sampling

All collection should be made as far from the ship itself as possible to minimize contamination from the ship. Plankton should be collected in regular 3/4 or 1- m plankton nets with either #6 or #10 nylon mesh netting. For hydrocarbon samples the cod end, at least, should be washed with clean solvent (e.g. ethanol) before use. Larger organisms can be collected by dip netting, jigging, hook and line, or long-lining. Mesopelagic organisms can be collected with Isaacs-Kidd mid-water trawls; benthic organisms with anchor dredges or beam trawls (Grice *et al.*, 1972). Care must be taken during handling to avoid contamination. Specimens and gear should be kept away from the ship's surface. Tools and storage containers should be cleaned before use (with pharmaceutical grade 95% ethanol for organics and doubly distilled nitric acid followed by distilled water for heavy metals). Handling and sorting gear must be of metal, glass, or enamel for organics and teflon or high density polyethylene for heavy metals.

B. Preservation

Samples should be rapidly placed in either glass or metal containers (organics) or plastic containers (heavy metals) and preserved frozen at -20°C.

References

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APPENDIX 1.

POTENTIAL OCEAN PLATFORMS FOR MARINE MONITORING AND RESEARCH PROGRAMS

Offshore Facilities

In addition to thousands of drilling platforms located on the continental shelf primarily in the Gulf of Mexico, off southern California, and off Alaska, there are 10 Coast Guard lightships and manned offshore towers that may be suitable for marine research monitoring. A list of the Coast Guard lightship and manned tower positions is as follows:

Name	Water Depth (ft)	Location
Buzzards Bay Tower	61	West Entrance, Buzzards Bay,Mass.
Ambrose Channel Tower	78	N. Y. Harbor entrance
Chesapeake Tower	63	12 Mi. E of Cape Henry, Va.
Diamond Shoal Tower	50	12 mi. ESE of Cape Hatteras, N.C.
Frying Pan Tower	46	25 Mi. SE of Cape Fear, S.C.
Portland Lightship	150	12 Mi. SE of Portland, Me.
Boston Lightship	108	12 Mi. SE of Boston, Mass.
Nantucket Lightship	192	48 Mi. SE of Nantucket Is.,Mass.
Five Fathom Lightship	90	16 Mi. SE of Cape May, N. J.
Colombia Lightship	210	Off Entrance to Colombia River

Coastal (Coast Guard) Stations

393 Coast Guard stations are located along the east, west, and gulf Coasts of the conterminous United States. These include 3 in Alaska, 3 in Hawaii, one in Puerto Rico, and one in the Virgin Islands. The stations operate boats varying in size from light skiffs to 52-foot motor lifeboats.

Ocean Station Vessels (OSV)

Ocean station vessels sail on 30-35 day patrols to occupy ocean stations, listed below (5 in the Atlantic, 1 in the Pacific). Schedules for station relief ensure that all ocean stations (with one exception) are continually manned. Ocean Weather Station (OWS) HOTEL is only occupied from August to March each year to assist in forecasting east coast winter storms. Approximately 20 days of on-station data collection can be realized on each patrol. Standard sections are routinely monitored *en route*

to and from ocean stations by each patrol vessel. These vessels range in length from 255 to 378 feet and have at least 3 berths for visiting scientists. The ships are equipped with NANSEN/STD, BT, and radiosondes and provide wet laboratory facilities.

OSV BRAVO	56°30'N	51°00'W
OSV CHARLIE	52°45'N	35°30'W
OSV DECTA	44°00'N	41°00'W
OSV ECHO	35°00'N	48°00'W
OSV NOVEMBER	30°00'N	140°00'W
OWS HOTEL	38°00'N	71°00'W

Merchant Ships

There are 2200 merchant ships which routinely provide meteorological data to the National Weather Service. Liaison is accomplished through port meteorological offices in major ports. Ocean sampling could be accomplished while the ship is underway.

Oceanographic and Fisheries Research Vessels

Approximately 15 NOAA and DOD research vessels are at sea at any one time conducting surveys for specific programs in geology, biology, physical oceanography, chemistry, and meteorology. Capability and cruise durations vary but are known in advance. Most have berths for scientists and have technicians aboard, as well as providing wet lab capability.

Patrol Vessels and Buoy Tenders (Coast Guard)

Also available are buoy tenders (including Tugs) which range in length from 65 to 213 feet. Most have scientist berthing and can be deployed for periods of 1 to 60 days; usually they are out less than a week at a time. There are also 79 patrol vessels available which vary in length from 82 to 95 feet and usually have one to two day deployments in near coastal waters.

Other

In addition, University research vessels may be available under contract or grant as well as government sponsored submersibles which may conduct special surveys. Other platforms not included in this list are environmental data buoys and environmental satellites.

Further information on these platforms should be obtained from the following:

Coast Guard Stations and vessels

Commandant
U. S. Coast Guard
407 "D" Street SW
Washington, D. C.

Research vessels

Oceanographer of the Navy
U. S. Navy Department
732 N. Washington Street
Alexandria, Virginia

Director
National Ocean Survey
National Oceanic and Atmospheric Administration
Rockville, Md. 20852

Merchant ships

Director, DATAC
National Weather Service
National Oceanic and Atmospheric Administration
Gramex Building
Silver Springs, Md.

APPENDIX 2.

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