METHODS OF ANALYSIS BY THE U.S. GEOLOGICAL SURVEY NATIONAL WATER QUALITY LABORATORY— PREPARATION PROCEDURE FOR AQUATIC BIOLOGICAL MATERIAL DETERMINED FOR TRACE METALS

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CONTENTS

CONVERSION FACTORS AND ABBREVIATED WATER-QUALITY UNITS

Multiply	By	To obtain
centimeter (cm)	3.94×10^{-1}	inch
gram (g)	3.53×10^{-2}	ounce, avoirdupois
liter (L)	2.64×10^{-1}	gallon
milligram (mg)	3.53×10^{-5}	ounce, avoirdupois
milliliter (mL)	2.64×10^{-4}	gallon
millimeter (mm)	3.94×10^{-2}	inch
microgram (µg)	3.53×10^{-8}	ounce, avoirdupois
microliter (µL)	2.64×10^{-7}	gallon
nanometer (nm)	3.94×10^{-8}	inch

Degree Celsius (°C) may be converted to degree Fahrenheit (°F) by using the following equation:

$$°F = 9/5 \ (°C) + 32.$$

Abbreviated water-quality units used in this report:

µg/L	microgram per liter
µg/mL	microgram per milliliter
µg/g	microgram per gram

Other abbreviations used in this report:

ac	alternating current
ASTM	American Society for Testing and Materials
BOD	biochemical oxygen demand
CV–AAS	cold vapor–atomic absorption spectrophotometry
DI	deionized
FEP	tetrafluoroethylene-hexafluoropropylene copolymer
GF–AAS	graphite furnace–atomic absorption spectrophotometry
hp	horsepower
Hz	hertz
ICP	inductively coupled plasma
ICP–MS	inductively coupled plasma–mass spectrometry
ICP–AES	inductively coupled plasma–atomic emission spectrometry
IDL	instrument detection limit
MDL	method detection limit
MPV	most probable value
MRL	method reporting limit
NAWQA	National Water-Quality Assessment program
NIST	National Institute of Standards and Technology
NWQL	National Water Quality Laboratory
SRM	standard reference material
USEPA	U.S. Environmental Protection Agency
V	volt
<	less than
≤	less than or equal to

Definitions:

MDL The method detection limit is defined as the minimum concentration of a substance that can be measured and reported with 99-percent confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte (U.S. Environmental Protection Agency, 1992).

MRL The method reporting limit is equal to the lowest reported concentration of an analyte by a given method.

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Abstract

A method for the chemical preparation of tissue samples that are subsequently analyzed for 22 selected trace metals is described. The tissue-preparation procedure was tested with three National Institute of Standards and Technology biological standard reference materials and two National Water Quality Laboratory homogenized biological materials. A low-temperature (85 degrees Celsius) nitric acid digestion followed by the careful addition of hydrogen peroxide (30-percent solution) is used to decompose the biological material. The solutions are evaporated to incipient dryness, reconstituted with 5 percent nitric acid, and filtered. After filtration the solutions were diluted to a known volume and analyzed by inductively coupled plasma–mass spectrometry (ICP–MS), inductively coupled plasma–atomic emission spectrometry (ICP–AES), and cold vapor–atomic absorption spectrophotometry. Many of the metals were determined by both ICP–MS and ICP–AES. This report does not provide a detailed description of the instrumental procedures and conditions used with the three types of instrumentation for the quantitation of trace metals determined in this study. Statistical data regarding recovery, accuracy, and precision for individual trace metals determined in the biological material tested are presented.

INTRODUCTION

The determination of trace-metal concentrations in biological tissue is generally accepted as a method to detect the presence of low-level trace metals in aquatic environments. Mechanisms by which trace metals become enriched in aquatic organism tissue have been discussed by Phillips (1980). The rationale for the use of aquatic tissue samples to ascertain the existence and uptake of waterborne trace metals for the U.S. Geological Survey National Water-Quality Assessment (NAWQA) program has been reported by Crawford and Luoma (1994).

This report describes the procedures used for chemical preparation of aquatic biological material and the subsequent instrumental analysis of the prepared samples for determining trace metals. Fish liver and freshwater clams (*Corbicula fluminea*, Asiatic clam) were the primary biological tissue types recommended for determining trace metals (Crawford and Luoma, 1994). To verify the sample-preparation and analysis procedures, two standard reference materials for biological tissues and one standard reference material for plants were obtained from the National Institute of Standards and Technology (NIST). These standard reference materials were used as surrogate samples in the verification because standard reference materials do not exist for the primary biological tissue types selected for the NAWQA program. Two biological tissue homogenates made from brown trout (*Salmo trutta*) livers and freshwater Asiatic clams (*Corbicula fluminea*) also were used. These two homogenates are not standard reference materials but were made at the National Water Quality Laboratory (NWQL) and used in this study because it was imperative to have samples that were representative of the type analyzed for the NAWQA program.

A low-temperature (85°C) nitric acid digestion procedure was used to

decompose the biological samples. The acid solutions were further oxidized by the careful addition of hydrogen peroxide (30-percent solution). The solutions then were carefully evaporated to near dryness, reconstituted with 5 percent nitric acid, and filtered to remove insoluble particulates. Sample-preparation procedures used in this study are similar to those reported by McDaniel (1992). The resultant solutions were analyzed for 21 trace metals by inductively coupled plasma–mass spectrometry (ICP–MS) or inductively coupled plasma–atomic emission spectrometry (ICP–AES), or both. Mercury was determined in these same solutions by cold vapor–atomic absorption spectrophotometry (CV–AAS).

This acid digestion procedure for determining 22 trace metals in biological material was developed by the U.S. Geological Survey for use in the NWQL and was implemented in October 1992. The method is the first to identify trace metals in samples of aquatic biological material at NWQL.

This report provides a detailed description of the method from sample preparation through calculation and reporting of results. Precision, recovery, and accuracy data for individual trace metals determined in the different biological materials tested are given.

ANALYTICAL METHOD

Inorganic Constituents and Parameter Codes:
Trace metals, biological, B-9001-95 (see table 1)

1. Scope and application

1.1 This method provides sample-preparation procedures for the nitric acid digestion of biological tissue for the subsequent determination of trace metals. The method is applicable to aquatic biological tissue and aquatic plant material only and is not applicable to

bivalve shells, bones, and sediment material contained in bivalves. The trace metals determined and the instrumental methods used to identify the trace metals in the NIST standard reference material and NWQL homogenate biological samples are listed in table 1.

2. Summary of method

2.1 Up to 20 g of wet tissue is placed into a tared 200-mL Pyrex beaker and weighed. The sample is placed in a constant-temperature oven and dried at 65°C over several days until constant weight is obtained. The sample is digested by first heating it with nitric acid and then by the careful addition of 30-percent hydrogen peroxide. Insoluble material (silica or sediment) is removed by filtration. Trace metals determined in the solutions are reported in micrograms per gram (μg/g) of dry-tissue weight.

3. Interferences and contamination

3.1 Chloride concentrations greater than 0.01 percent can cause analytical problems for ICP–MS used to determine trace metals by this digestion method. Therefore, hydrochloric acid was not used during the digestion procedure. The digested samples also were evaporated to near dryness and reconstituted with 5 percent nitric acid to remove all chloride present in the digested samples.

3.2 A clean working environment is required (Katz, 1984). The use of a class-100 clean bench during all manipulations, including filtration, drying of cleaned glassware, and acid rinsing of filters, is mandatory.

4. Apparatus and equipment

4.1 Graphite heating block

The graphite heating block is shown in figure 1. It is composed of five parts: (1) rectangular blocks of extruded graphite, (2) a silicone heating pad, (3) a

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Table 1. Instrumental analysis methods, codes, and registry numbers for trace metals

[NWQL, National Water Quality Laboratory; WATSTORE, Water Data Storage and Retrieval System; CAS, Chemical Abstract Service; μg/g, micrograms per gram; ICP–AES, inductively coupled plasma–atomic emission spectrometry; ICP–MS, inductively coupled plasma–mass spectrometry; CV–AAS, cold vapor–atomic absorption spectrophotometry]

Trace metal and method of analysis	NWQL code	WATSTORE code	Method code	CAS registry number
Aluminum (μg/g as Al)				7429-90-5
ICP–AES	6000	49237	A	
ICP–MS	6017	49237	B	
Antimony (μg/g as Sb)				7440-36-0
ICP–MS	6018	49246	B	
Arsenic (μg/g as As)				7440-38-2
ICP–MS	6019	49247	A	
Barium (μg/g as Ba)				7440-39-3
ICP–AES	6001	49238	A	
ICP–MS	6020	49238	B	
Beryllium (μg/g as Be)				7440-41-7
ICP–AES	6002	49248	B	
ICP–MS	6021	49248	A	
Boron (μg/g as B)				7440-42-8
ICP–AES	6003	49239	A	
Cadmium (μg/g as Cd)				7440-43-9
ICP–AES	6004	49249	C	
ICP–MS	6023	49249	A	
Chromium (μg/g as Cr)				7440-47-3
ICP–AES	6005	49240	A	
ICP–MS	6024	49240	B	
Cobalt (μg/g as Co)				7440-48-4
ICP–MS	6025	49250	A	
Copper (μg/g as Cu)				7440-50-8
ICP–AES	6007	49241	A	
ICP–MS	6026	49241	B	
Iron (μg/g as Fe)				7439-89-6
ICP–AES	6008	49242	A	
Lead (μg/g as Pb)				7439-92-1
ICP–AES	6009	49251	C	
ICP–MS	6028	49251	A	
Manganese (μg/g as Mn)				7439-96-5
ICP–AES	6010	49243	A	
ICP–MS	6029	49243	B	
Mercury (μg/g as Hg)				7439-97-6
CV–AAS	6046	49258	A	
Molybdenum (μg/g as Mo)				7439-98-7
ICP–AES	6011	49252	B	
ICP–MS	6030	49252	A	
Nickel (μg/g as Ni)				7440-02-0
ICP–AES	6012	49253	C	
ICP–MS	6031	49253	A	

Table 1. Instrumental analysis methods, codes, and registry numbers for trace metals—Continued

Trace metal and method of analysis	NWQL code	WATSTORE code	Method code	CAS registry number
Selenium (μg/g as Se)				7782-49-2
ICP–MS	6032	49254	A	
Silver (μg/g as Ag)				7440-22-4
ICP–AES	6013	49255	C	
ICP–MS	6033	49255	A	
Strontium (μg/g as Sr)				7440-24-6
ICP–AES	6014	49244	A	
Uranium (μg/g as U)				7440-62-2
ICP–MS	6036	49257	A	
Vanadium (μg/g as V)				7440-62-2
ICP–MS	6037	49465	A	
Zinc (μg/g as Zn)				7440-66-6
ICP–AES	6016	49245	A	
ICP–MS	6038	49245	B	

sheet of tempered glass, (4) a clear plastic cover (polyethylene), and (5) a solid-state power controller. The first four components of this heating system are highly resistant to acid fumes and are placed inside the laboratory hood. The solid-state power controller, which could be affected by acid fumes, is outside the laboratory hood.

NOTE 1: Do not use conventional metal hot plates because of contamination and safety concerns.

4.1.1 *Graphite blocks*—extruded, grade 890s; two pieces 1.3 by 25.4 by 38 cm; one piece 2.5 by 25 by 38 cm; one piece 5.1 by 25 by 38 cm; with twelve 5.7-cm holes cut all the way through, and one 0.64-cm hole halfway through for thermometer; Carbon/Graphite Group Inc. or equivalent.

4.1.2 *Heating mat*—two 15 by 38 cm, fiberglass reinforced silicone rubber; Curtin Matheson Scientific Inc.; 289-956 or equivalent.

4.1.3 *Power controller*—115 V, 60 Hz, with voltage level dial and on/off switch; Curtin Matheson Scientific Inc.; 272-924 or equivalent.

4.1.4 *Tempered glass*—common tempered glass at least 30 by 50 cm.

4.1.5 *Plastic cover*—polypropylene container at least 25 cm high by 38 cm wide by 61 cm long.

4.2 Clean bench

Labconco model 36125 class-100 laminar-flow clean bench or equivalent. Use for all manipu-lations that do not pose a safety hazard to the operator.

NOTE 2: Do not pour concentrated acids and other hazardous substances in a class-100 clean bench. Laminar-flow class-100 clean benches are designed to protect samples from contamination; they are not designed to protect the operator.

4.3 Water bath

4.3.1 *Polypropylene or Teflon container*—with a capacity of 4 to 8 L.

4.3.2 *Panel heater*—molded Teflon with no exposed metallic components; 20 cm long by 20 cm wide by 1 cm high; with a molded submersible power cord; Cole–Parmer Instrument Co., model H–03053–14 or equivalent.

Polyethylene Cover
(with Convection Vents)

Beaker Apertures
Thermometer Recess
Graphite Heating Block

Graphite Heating Block
Silicone Heating Pad
Tempered Glass
Insulating Platform

Air Flow

1 Unit = 3 Centimeters
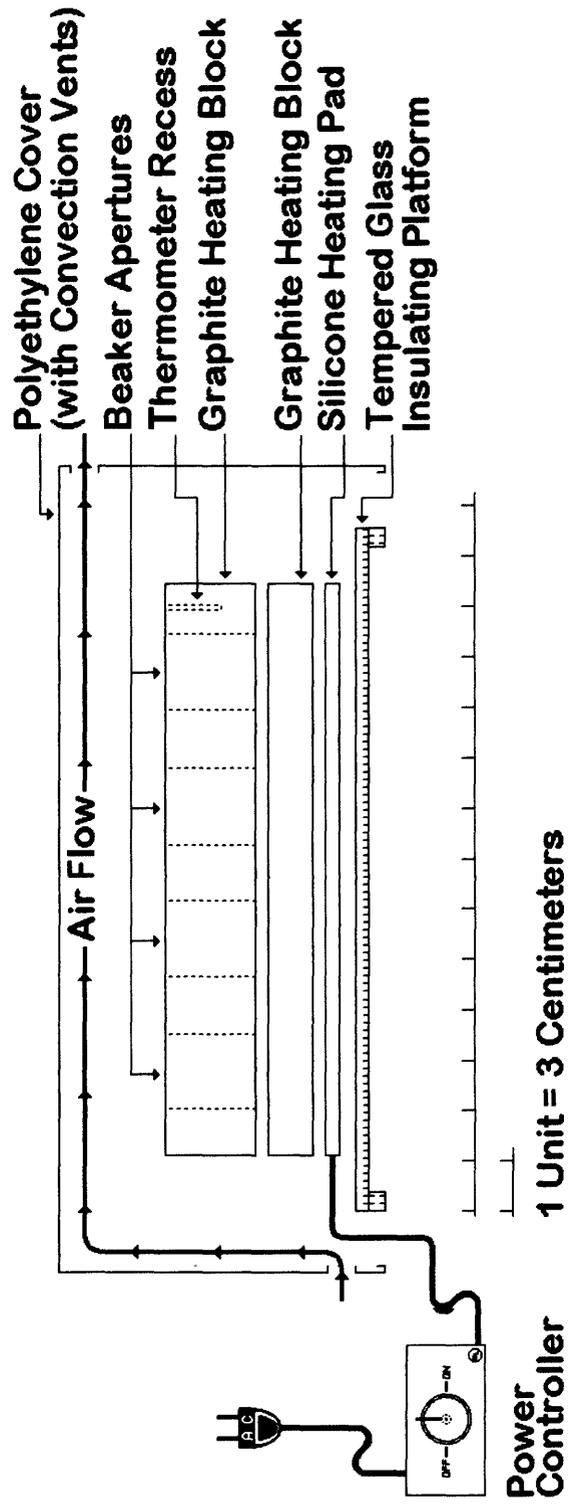
Power
Controller

Figure 1. Schematic showing graphite heating block.

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4.3.3 *Power controller*—115 V, 60 Hz, with voltage level dial and on/off switch; Cole–Parmer Instrument Co., model H–02604–00 or equivalent.

4.4 Disposable and nondisposable labware

Use Pyrex or quartz decomposition beakers for the acid digestion and heating steps. Use polyethylene, polypropylene, or Teflon FEP graduated cylinders, volumetric flasks, wash bottles, disposable pipet tips, and sample bottles.

4.5 Other equipment

4.5.1 *Disposable funnels with filters*—Whatman; polypropylene funnel with 47-mm filter; 250 mL total volume; grade 41 filter; Curtin Matheson Scientific Inc.; 279-244 or equivalent.

4.5.2 *Molded polypropylene drying baskets*—23 by 23 by 23 cm; Fisher Scientific; 14-965C or equivalent.

4.5.3 *Assorted sealable plastic storage boxes*—10- to 30-L Rubbermaid or equivalent.

4.5.4 *Fume hood*—acid resistant; interior covered with molded fiberglass; able to maintain 100 ft/min face velocity; 1/2 hp, 120-V ac motor; Labconco; 22473 or equivalent.

4.5.5 *Analytical balance*—Mettler PM600; 0 to 600-g range; capable of accurately weighing to 0.01 g; VWR Scientific; 11275-260 or equivalent.

4.5.6 *Deionized (DI) water system*—capable of Type I reagent water (American Society for Testing and Materials, 1995); production up to 2 L/min; Barnstead; D4741 or equivalent.

4.5.7 *Ultrasonic cleaner*—stainless-steel tank with a capacity of 5 L; Cole-Palmer; G-08852-00 or equivalent.

4.5.8 *Desiccator cabinet*—acrylic construction with built-in hygrometer to indicate relative humidity; Cole-Palmer; G-08897-00 or equivalent.

5. Reagents and standard reference materials

Check all reagents for purity prior to use.

NOTE 3: Do not use tap water for any part of this method, including cleaning procedures.

5.1 *Nitric acid, concentrated*—specific gravity 1.41; Baker Analyzed; 9598-33 or equivalent.

5.2 *Hydrogen peroxide solution*—30 percent; reagent grade; Baker; 2186-01 or equivalent.

5.3 *Water*—All references to water shall be understood to mean Type I reagent water (American Society for Testing and Materials, 1995).

5.4 *Standard reference biological material*—(1) Oyster tissue, No. 1566a; (2) Bovine liver, No. 1577A; and (3) Peach leaves, No. 1547; National Institute of Standards and Technology.

5.5 *Corbicula-tissue and Salmo-liver homogenate samples*—made for this study at the U.S. Geological Survey, NWQL .

NOTE 4: See Appendix A for procedures used to prepare the *Salmo*-liver and clam-tissue homogenates.

5.6 *Detergent*—general-purpose Alconox used for manual or ultrasonic cleaning; Cole-Palmer; G-17775-00 or equivalent.

5.7 *Desiccant*—indicating anhydrous calcium sulfate; Cole-Palmer; G-07193-15 or equivalent.

6. Labware cleaning

Minimize sample contamination by thoroughly cleaning all containers, pipet tips, and filtration apparatus that can contact the sample during processing or storage (Moody and Lindstrom, 1977; Karin and others, 1975).

6.1 Acid cleaning of Pyrex glassware

NOTE 5: Wear safety glasses or goggles and disposable plastic gloves during all cleaning procedures.

6.1.1 Sonicate all Pyrex beakers and watch glasses in 1 percent calgonite nonphosphate detergent for at least 1 hour in an ultrasonic cleaner to remove any organic films adhering to the glass surface.

6.1.2 Rinse the glassware thoroughly with DI water contained in a wash bottle or, if convenient, from a plastic tube connected to the delivery spigot of the DI water system.

6.1.3 Leach the rinsed glassware in a heated (50°C) sealable polypropylene tub filled with 5-percent nitric acid for at least 24 hours.

6.1.4 Remove the glassware from the tub, drain, and rinse glassware thoroughly with DI water after the first acid-soaking period.

6.1.5 Discard the 5-percent nitric acid in the tub, and rinse the acid-soaking tub with DI water.

6.1.6 Refill the acid-soaking tub with fresh 5-percent nitric acid and resoak the glassware at 50°C for at least 24 hours.

6.1.7 Remove the glassware from the soaking tub, drain, and rinse thoroughly with DI water.

6.1.8 Stack the wet glassware on clean plastic shelves in an operating class-100 clean bench.

6.1.9 When dry, store the glassware in acid cleaned, sealable plastic storage box to prevent dust accumulating on the clean glassware.

6.2 Acid cleaning of polyethylene bottles

6.2.1 Fill new plastic bottles to capacity with 5-percent nitric acid.

6.2.2 Recap the bottles and place in a heated water bath maintained at 50°C for 24 hours.

NOTE 6: Fill the water bath with ASTM Type I reagent water (American Society for Testing and Materials, 1995). The water level should be at least 5 cm below the neck of the plastic bottles. Polyethylene bottles filled with water have a density that is less than water. Therefore, a water-filled polyethylene bottle will fall over and float in water unless the water level is lower than the neck of the bottle. It is important to keep the bottles upright since it is not possible to ensure that all bottles will have airtight caps.

6.2.3 Remove the bottles from the water bath, discard the acid in the bottles, and fill with fresh 0.1-percent nitric acid.

6.2.4 Recap the bottles and replace them in the water bath for 24 hours at 50°C.

6.2.5 Discard this acid leachate after 24 hours of heating in the water bath, fill the bottles to overflowing with DI water, recap, and place in the heated water bath for 24 hours.

6.2.6 Discard the DI water in the bottles. Thoroughly rinse the bottles three times with DI water by completely filling them until they overflow. Rinse the caps in a flowing stream of DI water.

NOTE 7: Take care to ensure that fingers (even gloved fingers) do not come in contact with the inside of a cap during the rinsing process.

6.2.7 Dry the caps and empty bottles on plastic shelves in a class-100 clean bench.

6.2.8 Recap the bottles when dry and store in a sealable plastic storage box until needed.

6.3 Acid cleaning of disposable plastic pipet tips

6.3.1 Insert new pipet tips into a sealable widemouthed polypropylene container with at least 1-L capacity.

6.3.2 Fill the container to the top with 5-percent nitric acid and secure the lid.

6.3.3 Place the container in a heated water bath maintained at 50°C for 24 hours.

6.3.4 Discard the acid in the container after the initial heating period, refill with fresh 0.1-percent nitric acid, and replace in the heating bath for another 24 hours at 50°C.

6.3.5 Remove containers from the water bath and discard the leaching acid.

6.3.6 Thoroughly rinse the pipet tips in the container by filling the container with DI water, securing the lid, shaking the contents, and then discarding the DI water. Repeat this rinsing step three times.

6.3.7 Transfer the pipet tips into a polypropylene open-grid drying basket and place the basket in a class-100 clean bench.

6.3.8 Allow pipet tips to dry.

6.3.9 Insert the dry pipet tips into a resealable plastic bag, seal the bag, and place it in a sealable plastic storage box.

6.4 Acid cleaning of disposable plastic filter funnels

6.4.1 Clean all filter apparatus inside a class-100 clean bench.

6.4.2 Place new disposable filter funnels in plastic filter-funnel holders and fill rapidly to capacity with 5-percent nitric acid. Allow the acid to drain into empty glass beakers beneath each filter funnel. Repeat this cleaning step three times.

6.4.3 Rinse the filter funnels with DI water three times by rapidly filling the funnels and allowing them to drain.

6.4.4 Pour off any water (usually 2 to 3 mL) that remains in the filter funnel.

NOTE 8: Filter funnels can be cleaned immediately before they are needed, or they can be cleaned in advance and stored.

7.   Sample preparation

7.1 Receipt and storage of tissue samples

Biological material (packed with dry ice in an insulated cooler) must be frozen when received at the laboratory. Inspect all resealable bags to ensure the integrity of the bag and the condition of the samples. Record the condition of all compromised samples (torn bags or samples not frozen) in the sample preparation notebook. Inform the investigator who shipped the samples of their compromised condition. Double bag tissue samples in resealable polyethylene freezer bags and store in a freezer maintained at $\leq -10°C$ until sample preparation.

NOTE 9: Freeze/thaw cycles of biological tissue samples might cause biological cell structures to rupture and leak fluids (containing the trace metals of interest) into the containment bags. It is possible to maintain the inorganic chemical integrity of frozen biological samples for years provided the samples are not thawed and refrozen.

7.2 Sampling and subsampling aquatic biological material

7.2.1   Thaw biological samples in a refrigerator maintained at 0°C.

NOTE 10: Thawing at the freezing point of water will allow the entire sample to warm to a temperature conducive to cutting fish samples and shucking clam samples but will not allow the liquid portion of the soft tissue to leak.

7.2.2   Dissect biological tissue or shuck clams on a clean sheet of suitable plastic. It is not necessary to thaw and dissect the sample if the sample size is 20 g (wet weight). Shuck clams while still partially frozen to ensure that the intercellular fluids do not leak.

NOTE 11: The interior surface of large resealable food-storage bags is adequate for dissecting tissue.

NOTE 12: Serrated plastic blades (acid washed) are adequate for cutting partially thawed fish tissue and shucking partially thawed clams.

7.2.3   Place the tissue samples (approximately 20 g wet weight) into a clean preweighed Pyrex beaker and cover with a clean Pyrex watch glass.

7.3 Wet- and dry-weight determination

7.3.1   Record the wet weight of the samples and the beakers.

7.3.2   Cover the beakers and place them into a thermostatically controlled drying oven maintained at 65°C. Dry the samples for 24 hours.

NOTE 13: Large numbers of samples (greater than 10) will require several days to dry completely. They can be dried on weekends.

7.3.3   Remove the samples from the oven and place in a desiccator for 2 hours or until the samples reach room temperature.

7.3.4   Record the weight of the samples (remove the watch glass during the weighing) and replace the samples in the drying oven for another 24 hours.

7.3.5   Repeat the aforementioned weighing and drying steps until the weight-loss change is less than 10 percent of the previous tissue dry weights obtained.

7.4 Acid digestion of biological material

7.4.1   Place the covered beakers in an acid-resistant laboratory hood.

NOTE 14: The laboratory hood should have a plastic or fiberglass interior.

7.4.2   Carefully add 10 mL of concentrated nitric acid to the cool, dry tissue samples and wait several hours before adding more acid.

CAUTION—Some tissue samples are highly reactive to concentrated nitric acid and may froth out of the beaker.

7.4.3   Add an additional 30 mL concentrated nitric acid in 10-mL aliquots to the samples. Add each 10-mL aliquot only when any previous reaction has subsided.

7.4.4.   Allow the samples to digest at room temperature for 15 to 20 hours (overnight).

NOTE 15: After 24 hours, the tissue should be broken down and the acid

covered with at least 2.5 cm of foam. The solution should be dark red-brown.

7.4.5   Place the samples on a graphite block heater maintained at 45°C. Gently heat the samples for 10 hours at this temperature. Monitor the samples to ensure that the acid mixture does not foam out of the covered beakers.

NOTE 16: Nitrogen dioxide fumes are given off during this initial heating.

7.4.6   Raise the heating block temperature to 55°C and reflux the acid digestion mixture overnight.

NOTE 17: After this step in the digestion cycle, the dark brown solution containing only lipoidal material and insoluble particles should be evident.

7.4.7   Set the temperature of the heating block at 75°C and reflux the acid solution for 24 hours.

7.4.8   Remove the beakers from the heating block and allow the acid solutions to cool inside the fume hood.

NOTE 18: The acid solutions should be the color of a dark burgundy wine.

7.4.9   Add 5 mL of 30-percent hydrogen peroxide in 1-mL aliquots to the cool samples.

CAUTION—Hydrogen peroxide is extremely reactive when added to acid-solubilized organic compounds; the solution may boil out of the beaker. Generally, the first five 1-mL additions of hydrogen peroxide produce a vigorous exothermic reaction. Use extreme caution when adding each 1-mL aliquot of hydrogen peroxide; wait 10 to 15 minutes before adding each aliquot.

7.4.10   When no apparent reaction is evident, heat the acid solutions at 50°C. Watch the solutions carefully during this heating process. Do not leave the solutions unattended during this heating step.

NOTE 19: If the reaction becomes too vigorous, remove the beakers from the heating block and allow them to cool.

NOTE 20: As the acid digest oxidizes, the color of the solution will change from reddish to pale yellow and can become clear.

7.4.11   Add 15 mL of hydrogen peroxide in 5-mL aliquots or smaller volumes to the warm solution, depending on the reaction rate. Generally, 20 g (wet weight) of biological material will require 20 mL of hydrogen peroxide to oxidize the acid soluble organic compounds.

7.4.12   Insert the beakers into a hole of the graphite heating block maintained at 75°C. Reflux the solutions overnight. The solutions should be clear or pale yellow at the end of this heating period.

7.4.13   Place a plastic cover over the heating block containing the samples and evaporate the solutions to a volume of 1 mL or less. Samples can be evaporated to 1 mL or less in 24 hours.

NOTE 21: The plastic cover effectively converts the heating block to an oven. The temperature can be raised to 85°C during the day when liquid levels can be observed and lowered to 40°C at night. If the samples dry out at 40°C during the night, they will neither bake onto the glass surface nor spatter.

NOTE 22: The samples are evaporated to 1 mL or less to remove chloride as volatile hydrochloric acid.

7.4.14   Add 50 mL of 5-percent nitric acid to the samples.

7.4.15   Heat the solutions at 60°C for 30 minutes or until the lipoidal material melts and forms a droplet of clear oil on the surface of the acid.

7.4.16   Remove the beakers from the heat and allow the solutions to cool to room temperature.

NOTE 23: The lipoidal material will solidify into a small bead of wax-type material that is removed during the filtration step.

7.5. Filtration of acid-digested material

Filter the acid-digested solution prior to analysis. Filter all solutions inside a class-100 clean bench.

7.5.1 Place tared and acid cleaned 125-mL polyethylene bottles directly beneath the drain spouts of the filter funnels.

7.5.2 Pour the sample solutions into the filter funnels. Allow the solution to drain through the filter by gravity into the sample bottle.

7.5.3 Carefully rinse the sample beakers several times with a stream of 5-percent nitric acid delivered from a wash bottle. Pour the rinse solutions into the filter funnel.

7.5.4 Rinse the inside of the filter funnel with small (for example, 3 to 5 mL) quantities of 5-percent nitric acid when the volume inside the funnel is less than 5 mL. Wait 5 minutes between each addition of acid.

NOTE 24: Gravity filtration will stop when the volume inside the filter funnel is less than 3 or 4 mL. Adding small quantities of acid to the top of the sample solution will push the more concentrated sample through the filter.

7.5.5 Stop rinsing the filter funnel when the volume of acid delivered to the receiving bottle is approximately 90 mL.

7.5.6 Adjust the volume of each sample bottle to 100 mL by placing the bottle on a top-loading balance and adding 5-percent nitric acid until a weight of 103 g plus the original bottle tare weight is obtained. Record the weight for the sample solution because it is used to determine the volume of the solution.

NOTE 25: The density of 5-percent nitric acid is 1.03 g/mL.

7.5.7 Cap the sample bottles and place in a clean sealable plastic storage box until needed for analysis.

7.6 Remove an aliquot of the sample for mercury determination.

7.6.1 Pipet exactly 10 mL of the sample into a 250-mL BOD (biochemical oxygen demand) bottle.

NOTE 26: Pipet the subsample used for mercury analysis immediately after the final weight has been determined for the sample volume. Do not store samples to be analyzed for mercury in plastic for long periods because the mercury will adsorb onto the plastic container (Feldman, 1974).

7.6.2 Add 5 mL of concentrated nitric acid to the BOD bottle.

7.6.3 Add exactly 85 mL of DI water to the BOD bottle.

7.6.4 Seal the BOD bottle.

NOTE 27: Analyze the samples for mercury within 1 week after they have been pipeted into the BOD bottles.

8. Instrumentation and procedures

8.1 Use ICP–MS, ICP–AES, and CV–AAS to determine the concentrations of metals in the acid digested biological material. The metals determined by each analytical technique are listed in table 1.

8.1.1 Use the ICP–MS procedures described by Faires (1993). Two additional trace metals (arsenic and vanadium) were determined in biological material for this study that were not listed by Faires (1993). It was possible to determine these additional trace metals by ICP–MS for this study because chloride ions were removed by volatilization as

HCl from the solution during the acid-digestion procedure. Solutions that contain chloride ions cause isobaric polyatomic ion interferences (for example, OCl^+ and $ArCl^+$) for arsenic and vanadium. The sample acid digest was evaporated to minimize the chloride concentration, which in turn minimized the formation of polyatomic chloride ion interferences.

8.1.2 Use the ICP–AES procedures described by Fishman (1993). Two additional trace metals (aluminum and boron) have been determined in this study by ICP–AES (Struzeski and others, 1996).

8.1.3 Use the CV–AAS procedure to determine mercury as described by Fishman and Friedman (1989). Only 10 mL of the sample (10 percent of the total) was pipeted into a BOD bottle and acidified with 5 mL of concentrated nitric acid. The sample was diluted to 100 mL with DI water. The samples then were analyzed using procedures reported by Lobring and Potter (1992) and by Fishman and Friedman (1989).

9. Calculations

9.1 Calculate the wet weight and final dry weight for the samples, as follows:

Sample wet weight $\quad W_w = W_{wb} - W_b \quad$ (1)

Sample dry weight $\quad W_d = W_{db} - W_b \quad$ (2)

where W_w = sample wet weight, in grams;
W_{wb} = sample wet weight plus empty beaker weight, in grams;
W_b = empty beaker weight, in grams;
W_d = sample dry weight, in grams; and
W_{db} = sample dry weight plus empty beaker weight, in grams.

9.2 Convert concentrations of trace metals determined for the acid-digested tissue solutions to total mass quantity for the total sample. Convert this total mass quantity (in micrograms per sample) to a concentration of metal per gram of dry sample (in micrograms per gram). See equation 3 for this calculation:

$$C_t = (C_s \times V_s \times DF) / W_t \quad (3)$$

where C_t = metal concentration in the tissue sample, in micrograms per gram;
C_s = metal concentration in the acid-digested solution, in micrograms per liter;
V_s = volume of acid-digested sample solution, in liters;
DF = dilution factor of the analyzed acid digest; and
W_t = dry weight of tissue, in grams.

METHOD PERFORMANCE

The following samples were analyzed to evaluate the contamination, method detection limits, precision, accuracy, and recovery of the tissue-preparation and analytical procedures for the trace metals determined: (1) three NIST standard reference biological materials (oyster tissue SRM 1566a, bovine liver SRM 1577b, and peach leaves SRM 1547); (2) two NWQL prepared biological tissue homogenates (*Corbicula* tissue and *Salmo* livers); and (3) process blanks consisting of all reagents used in the tissue-digestion procedure. All metals determined in the *Corbicula*-tissue and *Salmo*-liver homogenate samples were spiked to evaluate possible interferences. Short-term (single-set) variation was compared to long-term (multiple-set) variation for precision, accuracy, and contamination.

Process blanks (short term)—The process blank results (mean and standard deviation) for a single set of eight blanks processed and analyzed as a group of samples are listed in table 2. Data from

three instrumental methods are reported in this table. For most of the metals, concentrations measured by ICP–AES are listed as less than the analysis method reporting limits (MRLs). (The MRL is defined in the front matter of this report.) Conversely none of the results for blank samples have been rounded at the ICP–MS method reporting limit. The ICP–MS mean and standard deviation results were calculated from uncensored data and account for the negative means for arsenic and selenium. Only aluminum, chromium, and zinc could be detected and measured by both methods. The results for these three metals are consistent for the two different methods. All of the results for both methods are consistent. In no case is a single metal concentration detected with ICP–MS greater than a less-than concentration for that same metal determined by ICP–AES.

The normal detection limit for mercury determined in a whole-water sample (sample size of 100 mL) by the CV–AAS procedure used at the NWQL is 0.1 µg/L. This detection limit is increased to 1 µg/L for tissue samples because only 10 percent of the original dissolved tissue sample was used for analysis.

Process blanks (long term)— Between December 3, 1992, and May 20, 1993, 25 process blanks were analyzed by ICP–AES, 28 by ICP–MS, and 31 by CV–AAS. These long-term blank studies are representative of the contamination possible for the complete method, including sample preparation and analysis. Long-term blanks determined by CV–AAS for mercury were all less than 1 µg/L. These mercury blank concentrations compare well with the single-day blanks (see table 2). The results for ICP–AES analysis are listed in table 3 and for ICP–MS analysis in table 4. Included in these tables for comparison are the tissue-process blanks from table 2, a single batch of samples analyzed on one day. There is no significant difference between the long-term and single-day results for metals determined by

Table 2. Single-day blank concentrations for the tissue-digestion procedure

[Eight blank replicates. µg/L, micrograms per liter; <, less than; --, data not calculable]

Trace metal	Mean concentration (µg/L)	Standard deviation (µg/L)
Aluminum[1]	47	22
Aluminum[2]	37	22
Antimony[2]	.04	.06
Arsenic[2]	-.03	.03
Barium[1]	<1	--
Barium[2]	.84	.33
Beryllium[1]	<.5	--
Beryllium[2]	.005	.019
Cadmium[1]	<1	--
Cadmium[2]	.26	.12
Chromium[1]	<5	--
Chromium[2]	5	2
Cobalt[2]	.08	.13
Copper[1]	<10	--
Copper[2]	.6	.1
Iron[1]	20.4	9.2
Lead[1]	<10	--
Lead[2]	.7	.8
Manganese[1]	<1	--
Manganese[2]	.68	.3
Mercury[3]	<1	--
Molybdenum[1]	<10	--
Molybdenum[2]	.3	.1
Nickel[1]	<10	--
Nickel[2]	2.2	.7
Selenium[2]	-.22	.38
Silver[1]	<1	--
Silver[2]	.01	.01
Strontium[1]	<.5	--
Uranium[2]	.001	.01
Vanadium[1]	<6	--
Vanadium[2]	.03	.01
Zinc[1]	4	1
Zinc[2]	3.5	.8

Method of analysis:

[1] Inductively coupled plasma–atomic emission spectrometry (ICP–AES).
[2] Inductively coupled plasma–mass spectrometry (ICP–MS).
[3] Cold vapor–atomic absorption spectrophotometry (CV–AAS).

ICP–AES, because the MRLs for most of the metals determined are greater than the concentrations of metals found in the process blanks.

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Aluminum, iron, and zinc had measurable concentrations in both the long-term and single-day blanks. Blank samples analyzed by ICP–MS could be evaluated for all the metals because of the lower detection levels (see table 4). Most of the metals determined by ICP–MS (18 total metals; table 4) in long-term studies have greater mean concentrations (10 metals) and greater standard deviations (14 metals) compared to the single-day samples. These are not unexpected results and indicate that long-term blanks are a better estimator of a true blank concentration than single-day measurements. However, single-day blanks are important and should be used to evaluate data produced during the analysis of samples in a single day.

Method detection limits for acid-digested tissue samples—The method detection limits (MDLs) for acid-digested tissue samples determined by ICP–AES and ICP–MS are listed in table 5. The MDLs were calculated using the U.S. Environmental Protection Agency (USEPA) MDL method (U.S. Environmental Protection Agency, 1992). These

MDLs are not normalized to the tissue weight but are given for the solution concentrations before conversion to the tissue dry weight concentration. The data used to calculated these MDLs were obtained from the long-term blank concentrations given in tables 3 and 4. For comparison, the method reporting limits (MRL) and instrument detection limits (IDL) for water samples determined by ICP–AES and ICP–MS are also listed in table 5. All ICP–AES MRL concentrations are given in the NWQL Services Catalog (Timme, 1995, p. 31–35) and ICP–MS MRL concentrations are obtained from Faires (1992, p. 4). The IDLs were determined at the NWQL and were calculated using the same procedure used to calculate the MDLs. Method blank concentrations were used to calculate the MDLs, however, low-level standards (approximately 5 times greater than the IDLs) were used to calculate the IDLs. MDLs for most trace metals determined by ICP–AES were not calculated because the method blank concentrations were less than the IDLs for this instrumental technique. However,

Table 3. Blank concentrations by inductively coupled plasma–atomic emission spectrometry measurements for the long-term and single-day studies

[μg/L, micrograms per liter; <, less than; --, data not available]

Trace metal	Long-term measurement, 25 blanks analyzed[1]		Single-day measurement, 8 blanks analyzed[2]	
	Mean concentration (μg/L)	Standard deviation (μg/L)	Mean concentration (μg/L)	Standard deviation (μg/L)
Aluminum	21	11	47	22
Barium	<1	--	<1	--
Beryllium	<.5	--	<.5	--
Cadmium	<1	--	<1	--
Chromium	<5	--	<5	--
Copper	<10	--	<10	--
Iron	11	5	20	9
Lead	<10	--	<10	--
Manganese	<1	--	<1	--
Molybdenum	<10	--	<10	--
Nickel	<10	--	<10	--
Silver	<1	--	<1	--
Strontium	<.5	--	<.5	--
Vanadium	<6	--	<6	--
Zinc	7	5	4	1

[1]Twenty-five tissue-process blanks were analyzed between December 3, 1992, and May 20, 1993.
[2]Eight tissue-process blanks were analyzed as a single batch on one day.

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Table 4. Blank concentrations by inductively coupled plasma–mass spectrometry measurements for the long-term and single-day studies

[μg/L, micrograms per liter]

Trace metal	Long-term measurement, 28 blanks analyzed[1]		Single-day measurement, 8 blanks analyzed[2]	
	Mean concentration (μg/L)	Standard deviation (μg/L)	Mean concentration (μg/L)	Standard deviation (μg/L)
Aluminum	21	14	37	22
Antimony	.09	.11	.04	.06
Arsenic	.1	.5	−.03	.03
Barium	.8	.4	.8	.3
Beryllium	.2	.2	.005	.019
Cadmium	.4	.2	.26	.12
Chromium	5	1	5	2
Cobalt	.02	.3	.08	.13
Copper	.7	1.5	.6	.1
Lead	.4	.3	.7	.8
Manganese	.6	.4	.68	.3
Molybdenum	.2	.1	.3	.1
Nickel	1.6	2.0	2.2	.7
Selenium	.6	1.5	−.22	.38
Silver	.10	.09	.01	.01
Uranium	.06	.20	.001	.01
Vanadium	.1	.5	.03	.01
Zinc	5.2	9.8	4	1

[1]Twenty-eight different process blanks were analyzed between December 3, 1992, and May 20, 1993.
[2]Eight different process blanks were analyzed as a single batch on one day.

Table 5. Method detection limits for acid-digested tissue blank samples analyzed by inductively coupled plasma–atomic emission spectrometry and inductively coupled plasma–mass spectrometry

[MRL, method reporting limit, IDL, instrument detection limit; MDL, method detection limit; μg/L, micrograms per liter; --, data not available]

Trace metal	Inductively coupled plasma–atomic emission spectrometry			Inductively coupled plasma–mass spectrometry		
	MRL (μg/L)	IDL (μg/L)	MDL (μg/L)	MRL (μg/L)	IDL (μg/L)	MDL (μg/L)
Aluminum	10	--	28.1	1	0.3	35
Antimony	--	--	--	1	.3	.3
Arsenic	--	--	--	--	.3	1.3
Barium	1	0.2	--	1	.2	.9
Beryllium	.5	.3	--	1	.2	.5
Cadmium	1	3	--	1	.4	.6
Chromium	5	4	3.8	1	.2	2.9
Cobalt	3	3	--	1	.2	.8
Copper	10	2	--	1	.2	3.6
Iron	3	3	12.1	--	--	--
Lead	10	30	--	1	.3	.7
Manganese	1	1	--	1	.1	1.1
Molybdenum	10	15	--	1	.2	.2
Nickel	10	13	--	1	2	5.0
Selenium	--	--	--	--	5	3.7
Silver	1	2	--	1	.2	.2
Strontium	.5	.5	--	1	.1	--
Uranium	--	--	--	--	.2	.5
Vanadium	6	3	--	1	.3	1.3
Zinc	3	7	12.2	1	.5	24

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MDLs for most trace metals determined by ICP–MS are larger than their corresponding IDLs.

The mercury MDL was determined by CV–AAS for spiked method blanks (1 μg/L) and calculated using the USEPA MDL method (U.S. Environmental Protection Agency, 1992). The calculated MDL concentration was 0.09 μg/L. The corresponding MRL concentration obtained from Timme (1995, p. 33) is 0.1 μg/L).

Analysis of Corbicula-tissue and Salmo-liver homogenates—The *Corbicula*-tissue and *Salmo*-liver homogenates used in these method-performance evaluations were prepared at the NWQL. The methods and conditions used to prepare these biological homogenates are listed in Appendix A.

Mean trace metal concentrations determined in *Corbicula*-tissue and *Salmo*-liver homogenates are listed in tables 6 and 7. All metals determined are reported in micrograms per gram dry weight. The mean, standard deviation, and percent relative standard deviation results listed in these two tables combine to show precision (seven replicates— individual homogenate samples) for preparation and the instrumental analysis. MDLs (ICP–MS analyzed samples only) listed in these tables were converted from microgram-per-liter concentrations listed in table 5 to microgram-per-gram concentrations for tables 6 and 7. The MDL concentrations listed in table 5 were multiplied by 0.1 (approximate final volume, in liters, of the acid-digested tissue sample) and divided by 2 (approximate dry weight, in grams, of a typical tissue sample). A comparison of MDL trace-metal concentrations to the trace-metal concentrations for *Corbicula*-tissue or *Salmo*-liver homogenates shows that each trace metal determined is not affected by blank concentrations to the same degree. For example, aluminum concentrations in *Corbicula*-tissue samples (table 6) are not affected by a MDL concentration of 1.7 μg/g, whereas,

aluminum concentrations in *Salmo* liver (table 7) are definitely affected by a MDL concentration of 1.7 μg/g. Most of the trace-metal concentrations determined are not affected by blank concentrations because they are at least a factor of 10 to 1,000 times larger than their respective blanks.

Spike recovery of trace metals added to Corbicula-tissue and Salmo-liver homogenate samples—Trace-metal spikes were added to the final acid-dissolved homogenate solutions to determine if matrix or chemical interferences, or both, were present for tissue samples analyzed by the three instrumental techniques used in this study. A preliminary analysis of all samples was made to determine the correct spike concentration to be added for each metal. As a general rule, all samples were spiked at a level approximately equal to each individual trace metal's concentration in the final analyzed sample. However, metals that could not be detected in an analyzed solution by ICP–AES, ICP–MS, or CV–AAS were spiked at levels approximately 10 times greater than their individual MDLs. Individual custom spike stock solutions were made for the different types of tissue samples analyzed. The concentration of the spike added to individual samples was determined by analyzing a 5-percent nitric acid blank spiked with the same stock solution as the sample. The calculation of percent spike recovery is given in equation 4:

$$\text{Percent } SR = [(SSC - USC)/SC]100 \qquad (4)$$

where percent SR = spike recovery, in percent;

SSC = spiked sample concentration, in micrograms per milliliter;

USC = unspiked sample concentration, in micrograms per milliliter; and

SC = spike concentration, in micrograms per milliliter.

Table 6. Trace-metal concentrations determined in *Corbicula*-tissue homogenate

[All concentrations are reported on a dry-weight basis. Seven replicates. μg/g, micrograms per gram; std. dev., standard deviation; MDL, method detection limit; <, less than; --, not calculable]

Trace metal	Mean concentration (μg/g)	Standard deviation (μg/g)	Percent relative std. dev.	MDL (μg/g)
Aluminum[1]	914	81	8.9	--
Aluminum[2]	1,025	53	5.2	1.7
Antimony[2]	.036	.007	19	.01
Arsenic[2]	1.9	.1	5.3	.06
Barium[1]	44	3	6.8	--
Barium[2]	50	2	4.0	.04
Beryllium[1]	<.25	--	--	--
Beryllium[2]	.073	.008	11	.02
Boron[1]	2.1	.2	10	--
Cadmium[1]	.81	.08	10	--
Cadmium[2]	.72	.04	5.6	.03
Chromium[1]	26	3	12	--
Chromium[2]	29	2	6.9	.14
Cobalt[2]	1.1	.03	2.7	.04
Copper[1]	35	5	14	--
Copper[2]	36	5	14	.18
Iron[1]	1,367	119	8.7	.6
Lead[1]	1.4	.4	29	--
Lead[2]	3.2	.2	6.2	.03
Manganese[1]	541	38	7.0	--
Manganese[2]	650	25	3.8	.05
Mercury[3]	<.1	--	--	.09
Molybdenum[1]	.83	.09	11	--
Molybdenum[2]	.63	.04	6.3	.01
Nickel[1]	1.22	.09	7.4	--
Nickel[2]	3.5	.1	2.9	.2
Selenium[2]	2.1	.5	24	.2
Silver[1]	.32	.05	16	--
Silver[2]	.37	.02	5.4	.01
Strontium[1]	30	2	6.7	--
Uranium[2]	.13	.02	15	.02
Vanadium[1]	1.7	.2	12	--
Vanadium[2]	1.8	.09	5.0	.06
Zinc[1]	167	12	7.2	--
Zinc[2]	152	7	4.6	1.2

Method of analysis:

[1] Inductively coupled plasma–atomic emission spectrometry (ICP–AES).
[2] Inductively coupled plasma–mass spectrometry (ICP–MS).
[3] Cold vapor–atomic absorption spectrophotometry (CV–AAS).

Table 7. Trace-metal concentrations determined in *Salmo*-liver homogenate

[All concentrations are reported on a dry-weight basis. Seven replicates. μg/g, micrograms per gram; std. dev., standard deviation; MDL, method detection limit; <, less than; --, not calculable]

Trace metal	Mean concentration (μg/g)	Standard deviation (μg/g)	Percent relative std. dev.	MDL (μg/g)
Aluminum[1]	2.9	0.5	17	--
Aluminum[2]	7.0	.6	8.6	1.7
Antimony[2]	.004	.003	75	.01
Arsenic[2]	.63	.08	13	.06
Barium[1]	.110	.004	3.6	--
Barium[2]	.13	.02	15	.04
Beryllium[1]	<.01	--	--	--
Beryllium[2]	<.01	--	--	.02
Boron[1]	.55	.05	9.1	--
Cadmium[1]	.07	.01	14	--
Cadmium[2]	.054	.006	11	.03
Chromium[1]	4.7	.5	11	--
Chromium[2]	4.7	.7	15	.14
Cobalt[2]	.088	.007	7.9	.04
Copper[1]	279	46	16	--
Copper[2]	294	50	17	.18
Iron[1]	272	12	4.4	.6
Lead[1]	.4	.1	25	--
Lead[2]	.55	.02	3.6	.03
Manganese[1]	4.2	.2	4.8	--
Manganese[2]	4.5	.2	4.4	.05
Mercury[3]	<.1	--	--	.1
Molybdenum[1]	1.1	.1	9.1	--
Molybdenum[2]	.87	.06	6.9	.01
Nickel[1]	<.5	--	--	--
Nickel[2]	.26	.03	12	.2
Selenium[1]	21	2	9.5	--
Selenium[2]	15	1	6.7	.2
Silver[1]	.14	.02	14	--
Silver[2]	.16	.01	6.2	.01
Strontium[1]	.51	.03	5.9	--
Uranium[2]	.017	.006	35	.02
Vanadium[1]	.33	.02	6.1	--
Vanadium[2]	.37	.03	8.1	.06
Zinc[1]	78	4	5.1	--
Zinc[2]	65	3	4.6	1.2

Method of analysis:

[1]Inductively coupled plasma–atomic emission spectrometry (ICP–AES).
[2]Inductively coupled plasma–mass spectrometry (ICP–MS).
[3]Cold vapor–atomic absorption spectrophotometry (CV–AAS).

Each percent *SR* calculation requires an analysis of the spike concentration, an unspiked sample, and a spiked sample. Mean percent spike recovery results for the analyzed *Corbicula*-tissue samples are listed in table 8 and for the *Salmo*-liver homogenates in table 9. The mean percent recovery results listed in these two tables are an average of seven individual homogenate samples, each spiked with an appropriate metal concentration. The quantity of spiked metal added to the samples analyzed by ICP–MS was a concentration factor 10 times less than that used for spiking samples analyzed by ICP–AES. Samples analyzed by ICP–MS are diluted by a factor of 10 before analysis. Percent recoveries ranged from 89 to 110 percent.

Trace metal recovery results for standard reference material (SRM)— Percent recovery results for NIST oyster tissue (SRM 1566a), bovine liver (SRM 1577a), and peach leaves (SRM 1547) are listed in tables 10, 11, and 12 (see Appendix B). Results for the metals determined in this study by the three analytical techniques are listed in these tables. Some of the metals determined in this study were not reported by NIST for one or all of the SRMs; therefore, a percent recovery could not be calculated for all of the metals determined in all the SRMs.

The percent recovery data for aluminum in NIST oyster tissue and peach leaves indicate the digestion procedure is not complete. The aluminum concentrations determined by ICP–MS and ICP–AES are in good agreement, indicating that the instrumental analysis is acceptable. This incomplete solubilization of aluminum in these two biological materials indicate the presence of highly acid-resistant minerals. It is probable that the aluminum in the NIST oyster tissue and in the homogenized *Corbicula*-tissue sample (see table 6) is caused by entrained sedimentary material that is not part of the soft tissue. The aluminum concentrations determined for the NIST bovine liver and the

Table 8. Percent recovery of trace metals added to samples of acid-digested *Corbicula*-tissue homogenate

[Seven replicates. μg/mL, micrograms per milliliter]

Trace metal	Amount added (μg/mL)	Mean percent recovery	Percent standard deviation
Aluminum[1]	10	94	8
Aluminum[2]	1	110	24
Antimony[2]	.01	99	3
Arsenic[2]	.01	103	6
Barium[1]	1	96	2
Barium[2]	.1	100	2
Beryllium[1]	.02	101	1
Beryllium[2]	.002	101	3
Boron[1]	.5	96	1
Cadmium[1]	.05	93	2
Cadmium[2]	.005	100	8
Chromium[1]	.5	93	4
Chromium[2]	.05	109	6
Cobalt[2]	.01	104	2
Copper[1]	1	98	2
Copper[2]	.1	105	13
Iron[1]	10	95	10
Lead[1]	.1	94	12
Lead[2]	.010	98	2
Manganese[1]	10	97	3
Manganese[2]	1	104	3
Mercury[3]	.002	99	2
Molybdenum[1]	.1	93	3
Molybdenum[2]	.01	103	4
Nickel[1]	.1	92	2
Nickel[2]	.02	104	7
Selenium[2]	.01	96	30
Silver[1]	.05	96	2
Silver[2]	.005	100	5
Strontium[1]	.5	96	4
Uranium[2]	.007	105	4
Vanadium[1]	.1	96	2
Vanadium[2]	.01	101	2
Zinc[1]	2	93	5
Zinc[2]	.1	102	6

Method of analysis:

[1] Inductively coupled plasma–atomic emission spectrometry (ICP–AES).

[2] Inductively coupled plasma–mass spectrometry (ICP–MS).

[3] Cold vapor–atomic absorption spectrophotometry (CV–AAS).

Table 9. Percent recovery of trace metals added to samples of acid-digested *Salmo*-liver homogenate

[Seven replicates. μg/mL, micrograms per milliliter]

Trace metal	Amount added (μg/mL)	Mean percent recovery	Percent standard deviation
Aluminum[1]	1	90	1
Aluminum[2]	.1	97.6	.8
Antimony[2]	.01	103	6
Arsenic[2]	.01	100	12
Barium[1]	.05	93.5	.5
Barium[2]	.005	105	3
Beryllium[1]	.010	100	2
Beryllium[2]	.001	102	6
Boron[1]	.5	95	1
Cadmium[1]	.05	94	2
Cadmium[2]	.005	102	5
Chromium[1]	.1	94	3
Chromium[2]	.01	101	5
Cobalt[2]	.01	96	3
Copper[1]	10	95	2
Copper[2]	1	96	3
Iron[1]	10	93.4	2
Lead[1]	.1	99	5
Lead[2]	.01	101	2
Manganese[1]	.1	94	2
Manganese[2]	.01	98	6
Mercury[3]	.002	99	3
Molybdenum[1]	.1	95.1	.8
Molybdenum[2]	.01	104	6
Nickel[1]	.1	97	2
Nickel[2]	.02	94	3
Selenium[2]	.025	102	7
Silver[1]	.05	89	4
Silver[2]	.005	94	3
Strontium[1]	.05	93.8	.7
Uranium[2]	.01	108	3
Vanadium[1]	.1	96	1
Vanadium[2]	.01	99	3
Zinc[1]	2	96	2
Zinc[2]	.15	99	3

Method of analysis:

[1]Inductively coupled plasma–atomic emission spectrometry (ICP–AES).

[2]Inductively coupled plasma–mass spectrometry (ICP–MS).

[3]Cold vapor–atomic absorption spectrophotometry (CV–AAS).

homogenized *Salmo* liver are low, indicating that this type of tissue does not concentrate aluminum.

These two types of tissue samples (for example, bovine liver and mollusks) are compared to illustrate a problem that might be inherent in using clam tissue from samples that have not been adequately depurated. If a large amount of sedimentary material is included in clam-tissue samples, the concentration of metals determined might be due partially to the sediment (Flegal and Martin, 1977; Chapman, 1985). This is important in bioaccumulation and biomagnification studies. The assumption that aluminum is not concentrated by clam tissue can be used to estimate the level of sediment in clam samples by measuring the concentration of aluminum. Even if aluminum is not completely solubilized with the present acid-digested procedure, it should be adequate to determine if large quantities of sedimentary material are present.

Stability of trace-metal solutions stored in polyethylene bottles—Biological tissue samples initially analyzed to determine accuracy and precision were reanalyzed many times during a 6-month period. Only metals determined by ICP–MS or ICP–AES were included in this study. Mercury was not included in this 6-month test because none of the biological materials tested had measurable concentrations, and the loss of low-level mercury stored in plastic bottles has been demonstrated by Feldman (1974). All four types of tissue samples used in the accuracy and precision studies were tested. The NIST peach leaves standard reference material was not tested. All tissue samples used in this 6-month stability study were prepared at the same time. Single samples of each of the four tissue types were analyzed by ICP–MS during the 6-month study. However, 6 to 10 of each of the four tissue types were analyzed by ICP–AES during the same period. It was possible to use a single sample for the ICP–MS analysis because

only 1 mL of sample was required for each analysis. Conversely, 10 mL of sample was required for the ICP–AES analysis. Therefore, these two analytical techniques were not used on the same individual samples.

Long-term trace-metal results for the ICP–MS analysis are listed in tables 13 through 16 (see Appendix B) for a single *Corbicula*-tissue homogenate (table 13), a single *Salmo*-liver homogenate (table 14), a single NIST oyster tissue (table 15), and a single NIST bovine-liver sample (table 16). Included in these tables are the appropriate single-day analytical results for NWQL homogenate samples or NIST standard reference materials. The NIST certified concentrations also are listed in tables 15 and 16 (see Appendix B).

Trace-metal results for the ICP–AES analysis of the *Corbicula*-tissue homogenates (10 samples) are listed in table 17. Of the *Salmo*-liver homogenates, 6 samples are listed in table 18; of the NIST oyster tissue, 9 samples are listed in table 19; and of the NIST bovine-liver tissue, 9 samples are listed in table 20. The appropriate single-day analysis of each of the different tissue types is included in each of these tables. Available NIST certified data also are listed in tables 19 and 20 (see Appendix B for tables 17–20).

SUMMARY OF METHOD

This acid-digestion method has been routinely used to prepare biological material for trace-metal analysis since October 1992. The acid digestates are analyzed for most trace metals by ICP–AES and ICP–MS. Many of the trace metals can be determined by either technique. Mercury, however, is determined only by CV–AAS. The following criteria are used to select the appropriate analytical technique (ICP–AES or ICP–MS) for reporting results: (1) Trace-metal concentrations— a factor of 10 larger than the detection limit for ICP–AES—are used because the results tend to be more precise; (2) trace-metal concentrations within a factor of 2 of the method detection limit for the ICP–AES technique are not reported; and (3) the ICP–MS results are used because of lower detection limits compared to the ICP–AES method.

Method performance was tested for contamination, method detection limit, precision, and accuracy.

(1) Contamination was tested for both short-term blanks (single-day sample set) and long-term blanks (multiple-day sample sets). Generally, long-term blank samples had larger mean concentrations and larger standard deviations than the short-term blank sample set.

(2) Method detection limits for acid-digested tissue samples were calculated using long-term digestion method blank concentrations. The MDLs for the complete digestion procedure are larger than the IDLs. The MDLs for the digestion method should be used to determine the most realistic detection limit for this procedure.

(3) Precision and stability studies indicate that measured metal concentrations are dependent on three factors: (1) analytical instrumentation used, (2) type of metal determined, and (3) concentration of the metal measured. As expected, metal concentrations measured near their MDLs are less precise for both single-day and long-term precision measurements compared to metal concentrations measured greater than their MDLs. At high metal concentrations (at least a factor of 10 greater than the MDLs), the precision of the total procedure generally is better than 10 percent. As the metal concentrations approach their respective MDLs, the relative percent standard deviations can increase to several hundred percent.

(4) Accuracy for the total method (digestion procedure and instrument analysis) was determined by analyzing NIST standard biological material and

spike additions to laboratory prepared *Corbicula*-tissue and *Salmo*-liver homogenates. Trace-metal recovery data for all of the NIST biological reference material indicate that the digestion procedure is adequate (if metals at or less than the MDLs are ignored) for most trace metals determined. For example, 14 of 15 metals (Al is the exception) determined in NIST oyster tissue with ICP–MS have recoveries between 80 and 111 percent (table 10). These same samples determined by ICP–AES have recoveries for 10 of 11 metals (Al is the exception) between 83 and 101 percent (table 10). Metal concentrations greater than the MDL determined by ICP–MS (12 metals) and ICP–AES (7 metals) in NIST bovine liver have recoveries between 80 and 117 percent (table 11). Spike recoveries for all metals determined by ICP–AES, ICP–MS and CV–AAS were between 89 and 110 percent (tables 8 and 9). These spike recoveries indicate that no matrix or chemical interferences are present for the two tissue samples tested for any of the instrumental techniques used for the analysis. Metal concentrations determined for field samples should always be compared to their respective MDL concentrations to determine if the concentration is real or an artifact of contamination. For example, bovine-liver and *Salmo*-liver aluminum concentrations determined in this study are similar to the microgram-per-gram MDL concentration.

The actual digestion procedure seems to be more precise than the ICP–MS analytical technique. The single-day precision data (based on multiple, individual homogenate or NIST standard reference material samples) have smaller standard deviations for most (64 times out of 72 possible comparisons) metal concentrations than the long-term precision data (on the basis of a single homogenate or NIST standard reference material sample). If the digestion procedure had a larger metal recovery variation than the ICP–MS analytical technique, then the individual metal concentration standard deviations for the single-day results would be larger than the

standard deviations for the long-term individual metal concentrations. Precision between the digestion method and the ICP–AES analytical technique cannot be compared because multiple samples were used for the single-day and the long-term precision studies.

REFERENCES CITED

American Society for Testing and Materials, 1995, Annual book of ASTM standards, section 11, Water: Philadelphia, v. 11.01, p. 122–124.

Chapman, P.M., 1985, Effects of gut sediment contents on measurements of metal levels in benthic invertebrates— A cautionary note: Bulletin of Environmental Contamination and Toxicology, v. 35, p. 345–347.

Crawford, J.K., and Luoma, S.N., 1994, Guidelines for studies of contaminants in biological tissues for the National Water-Quality Assessment program: U.S. Geological Survey Open-File Report 92–494, 69 p.

Faires, L.M., 1993, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of metals in water by inductively coupled plasma–mass spectrometry: U.S. Geological Survey Open-File Report 92–634, 28 p.

Feldman, Cyrus, 1974, Preservation of dilute mercury solutions: Analytical Chemistry, v. 46, no. 1, January 1974, p. 99–102.

Fishman, M.J., ed., 1993, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of inorganic and organic constituents in water and fluvial sediments: U.S. Geological Survey Open-File Report 93–125, 217 p.

Fishman, M.J., and Friedman, L.C., eds., 1989, Methods for determination of inorganic substances in water and fluvial sediments: U.S. Geological Survey Techniques of Water-Resources Investigations, book 5, chap. A1, 545 p.

Flegal, A.R., and Martin J.H., 1977, Contamination of biological samples by ingested material: Marine Pollution Bulletin, v. 8, p. 90–91.

Karin, R.W., Buono, J.A, and Fasching, J.L., 1975, Removal of trace elemental impurities from polyethylene by nitric acid: Analytical Chemistry, v. 47, no. 13, November 1975, p. 2296–2299.

Katz, S.A., 1984, Collection and preparation of biological tissues and fluids for trace element analysis: American Biotechnology Laboratory, v. 2, no. 4, December 1984, p. 24–30.

Lobring, L.B., and Potter, B.B., 1992, Method 245.6—Determination of mercury in tissues by cold vapor atomic absorption spectrometry, *in* Methods for the determination of metals in environmental samples: Cincinnati, Ohio, Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, p. 307–321.

McDaniel, William, 1992, Method 200.3—Sample preparation procedure for spectrochemical determination of total recoverable elements in biological tissues, *in* Methods for the determination of metals in environmental samples: Cincinnati, Ohio, Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, p. 25–32.

Moody, J.R., and Lindstrom, R.M., 1977, Selection and cleaning of plastic containers for storage of trace element samples: Analytical Chemistry, v. 49, no. 14, December 1977, p. 2264–2267.

Phillips, D.J.H, 1980, Quantitative aquatic biological indicators—Their use to monitor trace metal and organo-chlorine pollution: London, Applied Science Publishers Ltd., 488 p.

U.S. Environmental Protection Agency, 1992, Guidelines establishing test procedures for the analysis of pollutants (App. B, Part 136, Definition and procedures for the determination of the method detection limit): U.S. Code of Federal Regulations, Title 40, revised as of July 1, 1992, p. 565–567.

Struzeski, T.M., DeGiacomo, W.J., and Zayhowski, E.J., 1996, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory— Determination of dissolved aluminum and boron in water by inductively coupled plasma–atomic emission spectrometry: U.S. Geological Survey Open-File Report 96–149, 17 p.

Timme, P.J., 1995, National Water Quality Laboratory 1995 services catalog: U.S. Geological Survey Open-File Report 95–352, 120 p.

APPENDIX A

Preparation of *Salmo*-Liver and *Corbicula*-Tissue Homogenates

Salmo trutta (brown trout) liver was obtained from a local fish farm in Boulder, Colo. Livers were excised from fresh killed trout, placed in resealable plastic bags, and frozen. *Corbicula* were obtained from the S. Elkhorn Creek near Franklin County, Ky., and from the Potomac River near Navigation Bouy 66 by U.S. Geological Survey personnel during normal water-sampling trips. The *Corbicula* were collected by raking the mud, placing the collected *Corbicula* on a screen, and washing excess mud off the clams with local river water. *Corbicula* samples were not depurated prior to packaging in resealable plastic bags or aluminum foil. Samples were frozen, packed in an insulated container, and shipped to the NWQL by overnight mail.

Corbicula were thawed and shucked into a 4-L stainless-steel mixing bowl. A commercial hand mixer (Binds) fitted with four stainless-steel cutting blades was used to cut up and homogenize the thawed *Corbicula*. The mixture was cut and mixed for 30 minutes. The *Corbicula* mass then was strained through a stainless-steel screen mesh (2-mm screen mesh) into a second stainless-steel mixing bowl. About 20 percent of the material would not pass through the stainless-steel screen and was discarded. The *Corbicula* mass that passed through the screen was again homogenized with the hand mixer for 15 minutes. Approximately 20-mL quantities of homogenized *Corbicula* were transferred to precleaned 22-mL poly-ethylene screw-top vials. The transfer was facilitated by using a 20-mL disposable plastic pipet tip attached to an Oxford macrovolume pipet. Prior to using the tip, the end of the pipet tip was cut off with a razor blade so that the opening was approximately 10 mm. After every five vials were filled, the *Corbicula*-tissue homogenate was remixed with the hand mixer. Thirty vials were filled in this manner. The lids were secured to the vials and then placed in a freezer maintained at -10°C.

The *Salmo* liver was homogenized in an identical manner to that used for the *Corbicula* tissue. A total of thirty-five 20-mL samples was obtained from the *Salmo*-liver homogenization process. These samples also were placed in a freezer maintained at -10°C.

APPENDIX B

Tabular Data for Trace-Metal Concentrations
in Aquatic Biological Material

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Table 10. Percent recovery determined for National Institute of Standards and Technology oyster-tissue standard reference material

[All concentrations are reported on a dry-weight basis. Eight replicates. μg/g, micrograms per gram; NIST, National Institute of Standards and Technology; <, less than; --, data not calculable]

Trace metal	Mean concentration found (μg/g)	Standard deviation (μg/g)	Relative standard deviation (percent)	Mean recovery (percent)	Mean recovery, relative standard deviation (percent)
Aluminum[1]	96	7.0	7.3	47.4	3.5
Aluminum[2]	100	7.0	7.0	49.4	3.5
Aluminum (NIST)	202.5				
Antimony[2]	<.1	--	--	--	--
Antimony (NIST)	.1				
Arsenic[2]	12.5	.3	2.4	89.3	2.1
Arsenic (NIST)	14				
Barium[1]	1.57	.04	2.5	--	--
Barium[2]	1.64	.07	4.3	--	--
Beryllium[1]	.037	.005	14	--	--
Beryllium[2]	.006	.012	200	--	--
Boron[1]	9.8	.3	3.1	--	--
Cadmium[1]	4.2	.1	2.4	101	2.4
Cadmium[2]	3.9	.2	5.1	93.9	4.8
Cadmium (NIST)	4.15				
Chromium[1]	1.4	.1	7.1	97.9	7.0
Chromium[2]	1.3	.1	7.7	90.9	7.0
Chromium (NIST)	1.43				
Cobalt[2]	.46	.03	6.5	80.7	5.3
Cobalt (NIST)	.57				
Copper[1]	60	1	1.7	90.5	1.5
Copper[2]	62	2	3.2	93.5	3.0
Copper (NIST)	66.3				
Iron[1]	485	12	2.5	90.0	2.2
Iron (NIST)	539				
Lead[1]	<0.5	--	--	--	--
Lead[2]	.39	.04	10	105	10.8
Lead (NIST)	.371				

Table 10. Percent recovery determined for National Institute of Standards and Technology oyster-tissue standard reference material—Continued

Trace metal	Mean concentration found (μg/g)	Standard deviation (μg/g)	Relative standard deviation (percent)	Mean recovery (percent)	Mean recovery, relative standard deviation (percent)
Manganese[1]	11.1	0.3	2.7	90.2	2.4
Manganese[2]	11.4	.4	3.5	92.7	3.3
Manganese (NIST)	12.3				
Mercury[2]	<.1	--	--	--	--
Mercury (NIST)	.0642				
Molybdenum[1]	.4	.2	50	--	--
Molybdenum[2]	.21	.01	4.8	--	--
Nickel[1]	2.1	.3	14.3	93.3	13.3
Nickel[2]	2.5	.1	4.0	111	4.4
Nickel (NIST)	2.25				
Selenium[2]	2.4	.2	8.3	109	9.0
Selenium (NIST)	2.21				
Silver[1]	1.4	.2	14.3	83.3	11.9
Silver[2]	1.4	.2	14.3	83.3	11.9
Silver (NIST)	1.68				
Strontium[1]	9.8	.19	1.9	88.3	1.7
Strontium (NIST)	11.1				
Uranium[2]	.12	.01	8.3	90.9	7.6
Uranium (NIST)	.132				
Vanadium[1]	4.4	.1	2.3	94.0	2.1
Vanadium[2]	4.3	.1	2.3	91.9	2.1
Vanadium (NIST)	4.68				
Zinc[1]	833	24	2.9	100	2.9
Zinc[2]	827	21	2.5	99.6	2.5
Zinc (NIST)	830				

Method of analysis:

[1] Inductively coupled plasma–atomic emission spectrometry (ICP-AES).
[2] Inductively coupled plasma–mass spectrometry (ICP-MS).
[3] Cold vapor–atomic absorption spectrophotometry (CV-AAS).

Table 11. Percent recovery determined for National Institute of Standards and Technology bovine-liver standard reference material

[All concentrations are reported on a dry-weight basis. Eight replicates. μg/g, micrograms per gram; NIST, National Institute of Standards and Technology; <, less than; --, data not calculable]

Trace metal	Mean concentration found (μg/g)	Standard deviation (μg/g)	Relative standard deviation (percent)	Mean recovery (percent)	Mean recovery, relative standard deviation (percent)
Aluminum[1]	<1	--	--	--	--
Aluminum[2]	3	1	33.3	100	33
Aluminum (NIST)	3				
Antimony[2]	<.1	--	--	--	--
Antimony (NIST)	.003				
Arsenic[2]	.04	.01	25	80	20
Arsenic (NIST)	.05				
Barium[1]	.083	.005	6.0	--	--
Barium[2]	.09	.01	11	--	--
Beryllium[1]	<.1	--	--	--	--
Beryllium[2]	<.1	--	--	--	--
Boron[1]	3	2	67	--	--
Cadmium[1]	.49	.03	6.1	98	6
Cadmium[2]	.51	.03	5.9	102	6
Cadmium (NIST)	.50				
Chromium[1]	.91	.05	5.5	--	--
Chromium[2]	.56	.06	10.7	--	--
Cobalt[2]	.23	.01	4.3	92	4
Cobalt (NIST)	.25				
Copper[1]	150	6	4.0	94	4
Copper[2]	152	7	4.6	95	4
Copper (NIST)	160				
Iron[1]	181	7	3.9	98	4
Iron (NIST)	184				
Lead[1]	<0.5	--	--	--	--
Lead[2]	.13	.02	15	101	16
Lead (NIST)	.129				

Table 11. Percent recovery determined for National Institute of Standards and Technology bovine-liver standard reference material—Continued

Trace metal	Mean concentration found (μg/g)	Standard deviation (μg/g)	Relative standard deviation (percent)	Mean recovery (percent)	Mean recovery, relative standard deviation (percent)
Manganese[1]	10.0	0.4	4.0	95	4
Manganese[2]	9.9	.4	4.0	94	4
Manganese (NIST)	10.5				
Mercury[3]	<.1	--	--	--	--
Mercury (NIST)	.003				
Molybdenum[1]	4.1	.2	4.9	117	6
Molybdenum[2]	3.8	.2	5.2	109	6
Molybdenum (NIST)	3.5				
Nickel[1]	.4	.3	75	--	--
Nickel[2]	.67	.09	13	--	--
Selenium[2]	.7	.1	14	96	14
Selenium (NIST)	.73				
Silver[1]	.08	.01	12	205	26
Silver[2]	.044	.008	18	113	21
Silver (NIST)	.039				
Strontium[1]	.130	.006	4.6	96	4
Strontium (NIST)	.136				
Uranium[2]	.0007	.0014	200	--	--
Vanadium[1]	<.5	--	--	--	--
Vanadium[2]	.103	.007	6.8	84	6
Vanadium (NIST)	.123				
Zinc[1]	125	5	4.0	98	4
Zinc[2]	114	5	4.4	90	4
Zinc (NIST)	127				

Method of analysis:

[1]Inductively coupled plasma–atomic emission spectrometry (ICP-AES).
[2]Inductively coupled plasma–mass spectrometry (ICP-MS).
[3]Cold vapor–atomic absorption spectrophotometry (CV-AAS).

Table 12. Percent recovery determined for National Institute of Standards and Technology peach leaves standard reference material

[All concentrations are reported on a dry-weight basis. Eight replicates. μg/g, micrograms per gram; NIST, National Institute of Standards and Technology; <, less than; --, data not calculable]

Trace metal	Mean concentration found (μg/g)	Standard deviation (μg/g)	Relative standard deviation (percent)	Mean recovery (percent)	Mean recovery, relative standard deviation (percent)
Aluminum[1]	195	9	4.6	78	4
Aluminum[2]	196	8	4.1	79	3
Aluminum (NIST)	249				
Antimony[2]	<.1	--	--	--	--
Antimony (NIST)	.02				
Arsenic[2]	.13	.02	15	216	33
Arsenic (NIST)	.060				
Barium[1]	109	4	3.7	88	3
Barium[2]	131	6	4.6	106	5
Barium (NIST)	124				
Beryllium[1]	<.1	--	--	--	--
Beryllium[2]	.015	.004	27	--	--
Boron[1]	29	1	3.4	100	3
Boron (NIST)	29				
Cadmium[1]	<.1	--	--	--	--
Cadmium[2]	.04	.01	25	133	33
Cadmium (NIST)	.03				
Chromium[1]	1.1	.07	6.4	110	7
Chromium[2]	1.0	.04	4.0	100	4
Chromium (NIST)	1				
Cobalt[2]	.11	.01	9.1	--	--
Copper[1]	3.6	.1	2.8	97	3
Copper[2]	3.5	.1	2.9	95	3
Copper (NIST)	3.7				
Iron[1]	190	8	4.2	86	4
Iron (NIST)	220				

Table 12. Percent recovery determined for National Institute of Standards and Technology peach leaves standard reference material—Continued

Trace metal	Mean concentration found (μg/g)	Standard deviation (μg/g)	Relative standard deviation (percent)	Mean recovery (percent)	Mean recovery, relative standard deviation (percent)
Lead[1]	<.5	--	--	--	--
Lead[2]	.84	.05	6.0	96	6
Lead (NIST)	.87				
Manganese[1]	90	4	4.4	92	4
Manganese[2]	96	4	4.2	98	4
Manganese (NIST)	98				
Mercury[3]	<.1	--	--	--	--
Mercury (NIST)	.031				
Molybdenum[1]	<.5	--	--	--	--
Molybdenum[2]	.06	.01	17	100	17
Molybdenum (NIST)	.060				
Nickel[1]	.5	.2	40	72	29
Nickel[2]	1.9	.2	11	275	29
Nickel (NIST)	.69				
Selenium[2]	.13	.03	23	108	25
Selenium (NIST)	.120				
Silver[1]	<.1	--	--	--	--
Silver[2]	.012	.006	50	--	--
Strontium[1]	52	2	3.8	98	4
Strontium (NIST)	53				
Uranium[2]	.0095	.0029	30	63	19
Uranium (NIST)	.015				
Vanadium[1]	<.5	--	--	--	--
Vanadium[2]	.27	.01	3.7	73	3
Vanadium (NIST)	.37				
Zinc[1]	17.5	.8	4.6	98	4
Zinc[2]	17	1	5.9	95	6
Zinc (NIST)	17.9				

Method of analysis:

[1]Inductively coupled plasma–atomic emission spectrometry (ICP-AES).
[2]Inductively coupled plasma–mass spectrometry (ICP-MS).
[3]Cold vapor–atomic absorption spectrophotometry (CV-AAS).

Table 13. Long-term inductively coupled plasma–mass spectrometry precision measurements for one homogenized and acid-digested *Corbicula*-tissue subsample

[All concentrations are reported on a dry-weight basis. μg/g, micrograms per gram]

Trace metal	Long-term measurement, single homogenate[1]		Single-day measurement, eight homogenates[2]	
	Mean concentration (μg/g)	Standard deviation (μg/g)	Mean concentration (μg/g)	Standard deviation (μg/g)
Aluminum	1,139	123	1,025	49
Antimony	.06	.03	.036	.007
Arsenic	2.2	.4	1.9	.1
Barium	5.0	.4	5.2	1.5
Beryllium	.10	.03	.07	.01
Cadmium	.80	.09	.72	.04
Chromium	29	3	29.2	1.7
Cobalt	1.3	.2	1.10	.03
Copper	36	5	35.9	4.8
Lead	3.1	.3	3.2	.1
Manganese	652	55	650	23
Molybdenum	.65	.08	.62	.04
Nickel	4	1	3.5	.1
Selenium	3	1	2.1	.5
Silver	.37	.07	.37	.02
Uranium	.13	.05	.13	.02
Vanadium	1.8	.2	1.8	.1
Zinc	171	15	152	7

[1]Sample analyzed 14 times between December 3, 1992, and May 17, 1993.

[2]All eight individual homogenates were from the same batch used for the long-term measurements.

Table 14. Long-term inductively coupled plasma–mass spectrometry precision measurements for one homogenized and acid-digested *Salmo*-liver subsample

[All concentrations are reported on a dry-weight basis.
μg/g, micrograms per gram; <, less than; --, not calculable]

Trace metal	Long-term measurement, single homogenate[1]		Single-day measurement, eight homogenates[2]	
	Mean concentration (μg/g)	Standard deviation (μg/g)	Mean concentration (μg/g)	Standard deviation (μg/g)
Aluminum	6.5	0.9	7.0	0.6
Antimony	.010	.009	.004	.003
Arsenic	.8	.2	.6	.1
Barium	.15	.02	.11	.004
Beryllium	.01	.02	<.1	--
Cadmium	.06	.02	.1	.1
Chromium	4.1	.6	4.7	.6
Cobalt	.15	.09	.09	.01
Copper	337	23	294	46
Lead	.52	.05	.55	.02
Manganese	4.6	.3	4.5	.16
Molybdenum	.97	.06	.87	.05
Nickel	.6	.5	.26	.03
Selenium	17	2	14.6	1
Silver	.19	.02	.16	.01
Uranium	.018	.003	.017	.006
Vanadium	.5	.1	.37	.02
Zinc	81	5	64.7	2.4

[1]Sample analyzed 12 times between December 3, 1992, and May 17, 1993.

[2]All seven individual homogenates were from the same batch used for the long-term measurements.

Table 15. Long-term inductively coupled plasma–mass spectrometry precision measurements for one National Institute of Standards and Technology oyster-tissue standard reference material

[All concentrations are reported on a dry-weight basis. NIST, National Institute of Standards and Technology; SRM, standard reference material; μg/g, micrograms per gram; MPV, most probable value; <, less than; --, data not available]

Trace metal	Long-term measurement, single NIST SRMs analyzed[1]		Single-day measurement, eight NIST SRMs analyzed[2]		NIST certified concentrations	
	Mean concen-tration (μg/g)	Standard deviation (μg/g)	Mean concen-tration (μg/g)	Standard deviation (μg/g)	NIST MPV (μg/g)	95 percent confidence interval (μg/g)
Aluminum	99	7	100	7	202.5	12.5
Antimony	.03	.02	<.1	--	.01	--
Arsenic	13	1	12.5	.3	14.0	1.2
Barium	1.7	.1	1.6	.1	--	--
Beryllium	.08	.06	<.1	--	--	--
Cadmium	4.2	.3	3.9	.2	4.15	.38
Chromium	1.4	.3	1.3	.1	1.43	.46
Cobalt	.6	.1	.46	.03	.57	.11
Copper	66	5	62	2	66.3	4.3
Lead	.41	.04	.39	.04	.371	.014
Manganese	11.6	.8	11.4	.4	12.3	1.5
Molybdenum	.35	.05	.21	.01	1.68	.15
Nickel	2.5	.8	2.1	.3	2.25	.44
Selenium	3.0	.7	2.4	.2	2.21	.24
Silver	1.4	.2	1.4	.2	1.68	.15
Uranium	.13	.02	.12	.01	.132	.012
Vanadium	4.5	.3	4.3	.1	4.68	.15
Zinc	784	57	827	21	830	57

[1]Sample analyzed 19 times between December 3, 1992, and May 20, 1993.

[2]All eight individual NIST oyster SRMs were processed and analyzed as a single batch.

Table 16. Long-term inductively coupled plasma–mass spectrometry precision measurements for one National Institute of Standards and Technology bovine-liver standard reference material

[All concentrations are reported on a dry-weight basis. NIST, National Institute of Standards and Technology; SRM, standard reference material; μg/g, micrograms per gram; MPV, most probable value; <, less than; --, data not available]

Trace metal	Long-term measurement, single NIST SRMs analyzed[1]		Single-day measurement, eight NIST SRMs analyzed[2]		NIST certified concentrations	
	Mean concentration (μg/g)	Standard deviation (μg/g)	Mean concentration (μg/g)	Standard deviation (μg/g)	NIST MPV (μg/g)	95 percent confidence interval (μg/g)
Aluminum	2.2	0.3	2.6	1.6	3.0	--
Antimony	.02	.02	<.1	--	.03	--
Arsenic	.2	.2	.04	.01	.05	--
Barium	.11	.03	.09	.01	--	--
Beryllium	.05	.06	<.1		--	--
Cadmium	.5	.1	.5	.03	.5	0.03
Chromium	.8	.2	.6	.1	--	--
Cobalt	.29	.09	.23	.01	--	--
Copper	161	18	152	7	160	8
Lead	.14	.03	.13	.02	.129	.004
Manganese	10	1	9.9	.4	10.5	1.7
Molybdenum	3.9	.5	3.8	.2	3.5	.3
Nickel	.6	.4	.7	.1	--	--
Selenium	1.2	.6	.67	.13	.73	.06
Silver	.08	.05	.04	.01	.04	.01
Uranium	.003	.010	.0007	.0014	--	--
Vanadium	.2	.1	.103	.007	.123	--
Zinc	114	15	114	5	127	16

[1]Sample analyzed 14 times between December 3, 1992, and May 20, 1993.

[2]All eight individual NIST bovine-liver SRMs were processed and analyzed as a single batch.

Table 17. Long-term inductively coupled plasma–atomic emission spectrometry precision measurements for homogenized and acid-digested *Corbicula*-tissue subsamples

[All concentrations are reported on a dry-weight basis.
μg/g, micrograms per gram; <, less than; --, data not available]

Trace metal	Long-term measurement, ten homogenates analyzed[1]		Single-day measurement, eight homogenates analyzed[2]	
	Mean concentration (μg/g)	Standard deviation (μg/g)	Mean concentration (μg/g)	Standard deviation (μg/g)
Aluminum	950	59	914	75
Barium	46	2	43.6	2.5
Beryllium	.06	.02	<.1	--
Cadmium	.9	.2	.8	.1
Chromium	28.3	3.6	25.5	2.4
Copper	34	2	35	4
Iron	1,373	69	1,366	110
Lead	1.7	.5	1.4	.3
Manganese	549	22	541	35
Molybdenum	1.1	.2	.83	.09
Nickel	2.1	.6	1.2	.1
Silver	.44	.07	.3	.045
Strontium	32	1	30	1.9
Vanadium	1.9	.1	1.7	.2
Zinc	169	9	167	11

[1]Sample analyzed 19 times between December 8, 1992, and April 26, 1993.
[2]All eight homogenates were from the same batch used for the long-term measurements.

Table 18. Long-term inductively coupled plasma–atomic emission spectrometry precision measurements for homogenized and acid-digested *Salmo*-liver subsamples

[All concentrations are reported on a dry-weight basis.
μg/g, micrograms per gram; <, less than; --, data not available]

Trace metal	Long-term measurement, six homogenates analyzed[1]		Single-day measurement, eight homogenates analyzed[2]	
	Mean concentration (μg/g)	Standard deviation (μg/g)	Mean concentration (μg/g)	Standard deviation (μg/g)
Aluminum	3.0	0.9	2.9	0.5
Barium	.113	.005	.1	.004
Beryllium	<.01	--	<.01	--
Cadmium	.08	.03	.1	.02
Chromium	5.8	1.2	4.7	.5
Copper	301	16	279	42
Iron	281	13	272	12
Lead	.5	.1	.42	.1
Manganese	4.2	.2	4.2	.2
Molybdenum	1.3	.1	1.1	.1
Nickel	<.5	--	<.5	--
Silver	.17	.02	.1	.01
Strontium	.53	.03	.51	.02
Vanadium	.37	.03	.33	.02
Zinc	79	4	78	4

[1]Sample analyzed 12 times between December 8, 1992, and May 14, 1993.
[2]All eight homogenates were from the same batch used for the long-term measurements.

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Table 19. Long-term inductively coupled plasma–atomic emission spectrometry measurements for National Institute of Standards and Technology oyster-tissue standard reference material

[All concentrations are reported on a dry-weight basis. SRM, standard reference material; NIST, National Institute of Standards and Technology; µg/g, micrograms per gram; --, data not available; <, less than]

Trace metal	Long-term measurement, nine oyster SRMs[1]		Single-day measurement, eight oyster SRMs[2]		NIST certified concentrations	
	Mean concentration (µg/g)	Standard deviation (µg/g)	Mean concentration (µg/g)	Standard deviation (µg/g)	Mean concentration (µg/g)	95 percent confidence interval (µg/g)
Aluminum	.98	10	96	7	202.5	12.5
Barium	1.7	.1	1.57	.04	--	--
Beryllium	<.25	--	<.25	--	--	--
Cadmium	4.1	.2	4.2	.1	4.15	0.38
Chromium	1.4	.1	1.4	.1	1.43	.46
Copper	61	2	60	1	66.3	4.3
Iron	463	19	485	12	539	15
Lead	<.5	--	<.5	--	.371	.014
Manganese	10.6	.4	11.1	.3	12.3	1.5
Molybdenum	<.5	--	<.5	--	1.68	.15
Nickel	1.9	.5	2.1	.3	2.25	.44
Silver	1.5	.4	1.4	.2	1.68	.15
Strontium	9.9	.4	9.8	.2	11.1	1
Vanadium	4.5	.2	4.4	.1	4.68	.15
Zinc	817	38	833	24	830	57

[1] Sample analyzed 22 times between December 8, 1992, and May 20, 1993.
[2] All eight individual NIST oyster SRMs were processed and analyzed as a single batch.

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Table 20. Long-term inductively coupled plasma–atomic emission spectrometry measurements for National Institute of Standards and Technology bovine-liver standard reference material

[All concentrations are reported on a dry-weight basis. NIST, National Institute of Standards and Technology; SRM, standard reference material; μg/g, micrograms per gram; MPV, most probable value; <, less than; --, data not available]

Trace metal	Long-term measurement, nine NIST SRMs analyzed[1]		Single-day measurement, eight NIST SRMs analyzed[2]		NIST certified concentrations	
	Mean concen-tration (μg/g)	Standard deviation (μg/g)	Mean concen-tration (μg/g)	Standard deviation (μg/g)	NIST MPV (μg/g)	95 percent confidence interval (μg/g)
Aluminum	<0.2	--	2.0	2.0	3.0	--
Barium	.09	0.01	.1	.006	--	--
Beryllium	<.1	--	<.1	--	--	--
Cadmium	.51	.07	.49	.03	.5	0.03
Chromium	.9	.1	.9	.1	--	--
Copper	155	9	150	6	160	8
Iron	177	12	181	7	184	15
Lead	<.5	--	<.5	--	.129	.004
Manganese	9.8	.7	10	.4	1.5	1.7
Molybdenum	4.3	.3	4.1	.2	3.5	.3
Nickel	<.5	--	.4	.3	--	--
Silver	<.05	--	.08	.01	.039	.007
Strontium	.13	.01	.098	.0046	.136	.001
Vanadium	<.5	--	<.5	--	.123	--
Zinc	124	9	126	5	127	16

[1]Sample analyzed 33 times between December 8, 1992, and May 14, 1993.

[2]All eight individual NIST bovine-liver SRMs were processed and analyzed as a single batch.

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