Toxicants in Aqueous Ecosystems
A Guide for the Analytical and Environmental Chemist

T.R. Crompton

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With 19 Figures and 134 Tables

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Preface

Pollution of the ecosystem has always occurred to some extent or other. For example, over the whole of prehistory and still, to some extent, today, the eruption of volcanoes or the occurrence of fumaroles under the ocean has resulted in large-scale contamination of the ecosystem. Since the start of the Industrial Revolution, pollution of the ecosystem has obviously increased considerably and, despite efforts to control it, is still doing so.

Such inputs of pollution obviously include discharges of industrial and other waste and sewage directly into rivers and via coastal discharges. The emission of toxic substances into the atmosphere by factory smokestack emissions, incineration plants and fires is another major source of pollution, such emissions inevitably being washed out of the atmosphere by rain and then causing pollution of the oceans and land. Another input is the dumping of industrial and sewage wastes into the seas by ships.

Pollution is defined as a change in water quality that causes deleterious effects in the organism community or that makes the aesthetic quality of the water unacceptable. Contamination refers to the presence of potentially harmful substances at concentrations that do not cause harm to the environment.

It is becoming increasingly clear that the oceans and rivers, in particular, are not an unlimited reservoir into which waste can be dumped, and that control of these emissions is necessary if complete destruction of the environment is to be avoided. Heavy metals are particular offenders in this respect, as are organometallic compounds—whether the latter are discharged directly into the environment or whether (as has been shown in recent years) they are produced by the biological conversion of inorganic metallic contaminants such as lead, mercury and arsenic.

There are also many classes of organic pollutants that are encroaching upon the aquatic ecosystem. Organic pollutants—a subject that has been increasingly discussed in the public domain in recent years via the media—include crude petroleums, polyaromatic hydrocarbons, organochlorine and organophosphorus insecticides, polychlorinated biphenyls, chlorinated dioxins, chlorinated aliphatic and aromatic compounds, and nitrosamines. However, there are many thousands of possible organic pollutants, and only some of them have been studied in detail.

Once a toxic substance enters a river, it can cause damage to animal and plant life in the river, with possible implications for the survival of fish and invertebrates and also for the health of the humans who eat these creatures. Many rivers serve as inputs to potable water treatment plants and consequently so there are further health implications for humans and animals that drink the water. River waters often carry the pollutants to the oceans, where they are added to by the pollutants in coastal discharges, atmospheric fallout and shipboard dumping. Again, the survival of animal populations and the health of humans become major considerations. Pollutants that discharge directly onto land, including sewage and domestic and industrial waste, are inevitably washed by rain to a watercourse and eventually end up in the sea.

Regulations for controlling the input of pollutants into the environment are slowly being introduced internationally, but much remains to be done.

It is the purpose of this book to describe in detail methods for the determination of all types of pollutants—inorganic, organic and organometallic—in fish, crustacea and other marine creatures, as well as in weeds, plants, phytoplankton, algae and so on (Chaps. 1–3).

The levels of pollutants that occur in these depends on the levels of pollutants that occur in the water in which they live, and in the case of bottom-feeding fish and crustacea on the pollutant levels that occur in sediments. Sediments in the beds of rivers and in the oceans adsorb many toxicants from the water in such amounts that the concentrations of toxicants in the sediment are many times—in some instances up to a million times—higher than in the surrounding water. Analysis of sediments is therefore a useful means of assessing the pollutant levels in water over a period of time, and is related to ill health or mortality of creatures living in the water. A review is given in Chap. 4 of the levels of inorganic, organic and organometallic toxicants found in such sediments in samples taken all over the world, and an attempt is made to correlate contaminant levels with the health of creatures. This aspect is fully discussed in the author's previous books [1–5].

Chapter 4 reviews the levels of metals, organometallics and organics found in the tissues of various types of fish and invertebrates as well as in phytoplankton and weeds taken at various sites throughout the world. In addition, results are reported for the levels of metals found in the organs of these creatures, as in many instances enhanced metal levels occur in particular organs, and this allows the cause of death to be identified. In particular, polyaromatic hydrocarbons, chlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin are discussed.

In Chaps. 5–9, examples of the effect of dissolved metallic toxicants on freshwater organisms and seawater organisms are discussed. Using published LC_{50} and maximum safe concentration (S_x) data, it is possible to draw-up 'at risk' tables for each type of creature. One can then use these to compile a list of creatures from any particular water with any particular

composition that will either suffer ill health or will die. Examples of clean and dirty rivers are discussed.

These chapters discuss available toxicity data describing the effects of various types of pollutants on fish and invertebrates. These include studies on nonsaline and saline waters and cover all the toxic metal pollutants, organic pollutants and organic compounds of arsenic, lead, mercury and tin.

The exposure of creatures to known concentrations of toxicants for stipulated periods of time enables the toxicity of the pollutant to be established, as measured by the relationship between the concentration and the time taken for 50% of the creatures to die (LC_{50}), or to experience adverse effects, i.e., LE_{50}. Such water analysis-based methods for assessing the effects of pollutants are discussed in Chap. 10. A further method of assessing the toxicity of pollutants is based on relating the composition of the water in which the creatures live to the concentration of the toxicant found in the animal tissue, or, better still, in a particular organ of the animal in which the toxicant concentrates preferentially. Such data can be related to the water composition and the condition of the animal in terms of ill health or mortality. These methods are reviewed in Chap. 11.

This book is essential reading for all analytical chemists, environmentalists and toxicologists working in the field.

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1 Analysis of Fish

1.1 Cations

1.1.1 Aluminium

Harvey (Clyde River Purification Board, unpublished report) has described a method for the determination of aluminium and calcium in fish gills.

After washing with distilled water, the gill filaments were cut away from the bony gill arches, placed in acid-washed glass petri dishes and dried at 105 °C. The dry material was weighed and digested with concentrated nitric acid. The digest was filtered, the residue washed, and filtrate made up to the 25 ml. Calcium was determined by atomic absorption using background correction and an air/acetylene flame. Lanthanum chloride was added as a releasing agent to all solutions in order to give a concentration of 0.1% lanthanum in the final solutions. The calcium standards used were 2, 5 and 10 mg/l. Aluminium was determined using a nitrous oxide/acetylene flame, 309.3 nm wavelength, background correction and standards of 5, 10, and 20 mg/l for absorption. A wavelength of 396.2 nm, along with standards of 1, 3 and 5 mg/l, were used for emission. A slit of 0.3 was used and potassium chloride was added as an ionisation buffer to all solutions to give a final concentration of 0.1% potassium. Flame conditions were the same for both modes.

Heydorn et al. [1] have discussed the determination of aluminium in fish gills using neutron activation analysis and inductively coupled plasma mass spectrometry. Considerable contamination was obscured with both methods, which required the samples to be handled using a clean bench and super-pure reagents.

Ranau et al. [2] used graphite furnace atomic absorption spectrometry (AAS) after pretreatment with microwave-activated oxygen plasma to determine aluminium.

1.1.2 Arsenic

Maher [3] has described a procedure for the determination of total arsenic in fish. The sample is first digested with a mixture of nitric, sulfuric and perchloric acids. Then arsenic is converted into arsine using a zinc reductor column, the evolved arsine is trapped in a potassium iodide–iodine solution, and the arsenic determined spectrophotometrically at 866 nm as the arsenomolybdenum blue complex. The detection limit is 0.3 mg/kg dry fish and the coefficient of variation is 5.1% at this level. The method is free from interferences by other elements at levels normally found in fish. Values of 9.7 ± 0.3 and 13.2 ± 0.4 mg/kg obtained for NBS reference waters SRM 1S71 and SRM 1566, respectively, were in good agreement with the nominal values of 10.2 ± and 13.4 ± mg/kg. Spiked sampled crayfish gave 98 – 100% recovery of arsenic by this procedure.

Agemian and Thomson [4] have described a semi-automated AAS method for the determination of arsenic in wet homogenised fish tissue. A combination of nitric, perchloric and sulfuric acids is used to dissolve high fat fish tissues at 140 – 180 °C in a glass tube. Extracts are then analysed by reduction to arsine with sodium borohydride, followed by AAS with quartz tube atomisation. Average recoveries of arsenic(III), arsenic(V), *p*-arsalinic acid, benzene arsenic acid, methylarsenic, and triphenylarsine oxide obtained using this procedure were between 90 and 102%. Arsenic found in a NBS standard bovine liver (SRM 1577) reference sample was 500 ± 10 mg/kg against a certified value of 550 ± 5 mg/kg. Arsenic levels found in fish samples ranged from 0.26 to 0.44 mg/kg, determined with a coefficient of variation of 7 – 15%.

Brooke and Evans [5] described two methods for the digestion of fish samples prior to determination of arsenic down to 0.02 mg/kg by hydride generation AAS.

The first method involves separation of the inorganic arsenic by distilling it from 6.6 N hydrochloric acid. The second method involved chelation and extraction of inorganic arsenic after sample dissolution in sodium hydroxide solution, with subsequent back-extraction and oxidation. In both methods the arsenic concentration is measured after hydride generation by AAS with atomisation in a flame-heated silica tube; in the first method the solution contains arsenic(III), and in the second the solution contains arsenic(V). Results obtained by both methods are in agreement over a range of samples. The distillation method is favoured for reasons of efficiency and economy in time.

Hydrochloric Acid Digestion

Weigh 5 grams of a representative wet fish sample (2 g of dry sample) into a 125 ml pear-shaped flask. Add 5 ml of water and 1 ± 0.1 g of iron(II) sulfate heptahydrate. Through the Bethge trap, add 50 ml of hydrochloric

acid (3 + 2) and reflux the reaction mixture for ten minutes. Close the tap in the Bethge trap and collect the first 50 ml of distillate over a period of 30 minutes. Cool and transfer into a 100 ml calibrated flask, washing with water, to give 100 ml of a colourless solution free from suspended solids. Reagent blank solutions should be obtained from hydrochloric acid (3 + 2) in an identical manner.

Sodium Hydroxide Digestion

Place 2 g of a representative wet fish sample (1 g of dry sample) into a 150 ml conical flask, add 10 ml of sodium hydroxide reagent and heat on a boiling water bath for 20 minutes. Cool, cautiously add 35 ml of hydrochloric acid (1 + 3), and cool further. Transfer the solution into a separating funnel using 5 ml of water for washing, add 2 ml of ammonium pyrrolidone dithiocarbamate solution, and mix thoroughly. Extract with 10 ml of 4-methylpentan-2-one, shaking for 2 minutes, allow to stand for 5 minutes or until separation is complete, and run off the solvent into another separating funnel. Repeat the extraction with the addition of ammonium pyrrolidone dithiocarbamate reagent, and finally extract with 10 ml of 4-methylpentan-2-one. To the combined solvent extracts add 10 ml of nitric acid (1 + 7) and shake for 2 minutes. Repeat this extraction twice and combine the extracts in a beaker. Add 5 ml of sulfuric acid (1 + 1) and boil until white fumes are evolved. Cool, add 10 ml water, re-heat to fuming, and repeat. Dilute to 50 ml. Reagent blank solutions should be obtained in an identical manner.

Recoveries of inorganic arsenic in spiked fish samples were 80 – 94% for arsenic(III) and 75 – 88% for arsenic(V). Total and organic arsenic levels found by both methods in some fish samples are given in Table 1.1.

Goulden et al. [6] have described a semi-automatic procedure for the determination of arsenic in nitric–perchloric acid digests of fish. Arsenic is determined using inductively coupled argon plasma (ICAP) excitation using a plasma power of 1400 W.

Beauchemin et al. [7] identified and determined the arsenic species present in dogfish muscle reference material (DORM-1). The arsenic species present were identified using electron impact mass spectrometry (IMS), thin-layer chromatography (TLC) and high-performance liquid chromatography

Table 1.1. Arsenic determination in fish. From [5]

Fish	Total arsenic[a] mg/kg	Inorganic arsenic mg/kg		Inorganic arsenic as % of total arsenic
		HCI digestion	NaOH digestion	
Herring	1.1	0.03	0.04	3.6
Haddock	2.6	0.03	0.02	0.8
Tuna	2.9	0.17	0.13	4.5

[a] Obtained by dry ashing

(HPLC)/ICA spectrometry. Determination was by the latter technique and graphite furnace atomic absorption spectrometry (GF-AAS). Arsenobetine was the major arsenic species in the methanol/water fraction (84% of the total arsenic). Arsenic(III), arsenic(V), monomethylarsonic acid, dimethylarsinic acid and arsenocholine constituted 4%. The total arsenic concentration was 18.7 mg/kg. The detection limit was 0.3 ng arsenic.

High-performance liquid chromatography accompanied by ICP optical emission spectrometry and hydride generation quartz furnace AAS has been used [8] to determine six arsenic species in marine organisms.

Branch et al. [9] and Le et al. [10] directly coupled HPLC to ICP–MS to determine arsenic in fish.

Yun-Kai-Lu et al. [11] carried out a simultaneous determination of traces of arsenic and cadmium in biological samples using hydride generation–double channel atomic furnace spectrometry.

1.1.3 Cadmium

Blood and Grant (private communication) determined cadmium in fish tissue using flameless AAS at 228.8 nm using a tantalum ribbon.

Fish samples were digested by one of two techniques: either 1 ml of the acid mixture (3 parts concentrated nitric acid by volume: 1 part concentrated sulfuric acid: 1 part concentrated perchloric acid) was placed together with a weighed sample (1 to 100 mg) in a covered 3.5 ml polypropylene test tube for 2 hours at 74 °C in a water bath and, finally, diluted to 25 ml; or 1 ml of concentrated nitric acid was added to the sample, which was heated for 15 minutes at 80 – 90 °C followed by the addition of 1 ml of 10% hydrogen peroxide and heating for an additional 15 minutes.

Mean recoveries obtained on the NBS SRM 1577 reference bovine liver sample with an authenticated cadmium content of 0.27 ± 0.04 mg/kg were 96.2% (nitric–perchloric–sulfuric acid digestion) and 84.8% (nitric acid–hydrogen peroxide digestion). In general, higher results were obtained by the sulfuric-nitric acid digestion procedure. The higher mean levels (13.1 – 5.6 mg/kg) of cadmium in wet blue gill tissue were found in kidney, gut, heart, gill, and liver and the lowest levels (0.14 – 1.7 mg/kg) in muscle, skin and bone.

In a series of papers, Sperling [12–14] studied the application of flameless GF–AAS to the determination of cadmium in complex matrices resulting from the digestion of fish and other biological materials. Organic material in the sample is destroyed before atomisation by digestion with ammonium peroxydisulfate, thereby avoiding loss of volatile cadmium, which would occur in ignition methods at temperatures exceeding 420 °C [12]. Cadmium was then extracted from the digest with a saturated solution of ammonium pyrrolidone dithiocarbamate in carbon tetrachloride [13, 14], and the cadmium in the lower layer determined by flameless GF-AAS.

Poldoski [15] used a molybdenum- and lanthanum-treated pyrolytically coated graphite tube for the GF–AAS determination of cadmium at 228.8 nm in nitric acid perchloric acid digests of fish tissue. Molybdenum and lanthanum help reduce chemical interferences and interference from uncompensated background signals during analyte atomisation.

Digestions were carried out on 0.6 g of dry fish using 10 ml concentrated nitric acid and 2 ml perchloric acid. After digestion was complete, the residue was dissolved in 10 ml 0.2% w/v nitric acid and stored in Nalgene bottles.

Cadmium spiking recovery experiments were carried out on fish tissue samples on an authenticated reference sample (NBS SRM 1577 bovine liver) under specified conditions of analysis. The determination of cadmium content on a NBS SRM reference fish sample (0.31 $\pm$ 0.05 mg/kg) is in good agreement with the nominal value (0.27 $\pm$ 0.04 mg/kg). In addition, 0.038 mg/kg recoveries of cadmium in the fish samples were 91 – 97%. Down to 0.2 pg cadmium could be determined in the injected portion of the sample. Cadmium contents determined by this technique were in good agreement with those obtained by anodic scanning voltammetry.

1.1.4 Cobalt

Kiriyama and Kuroda [16] applied their combined ion-exchange spectrophotometric procedure to the simultaneous determination of cobalt and vanadium in cutlass fish. In this procedure, the sample is dry ashed at 420 °C, the ash (ca. 0.5 g) is decomposed with a mixture of perchloric, nitric, and hydrofluoric acids, and is finally taken up in hydrochloric acid. The metals are adsorbed by anion exchange on an Amberlite CG 400 (SCN-) column from a dilute ammonium thiocyanate–hydrochloric acid solution. The adsorbed vanadium and cobalt are separated chromatographically by elution with 12 mol/l hydrochloric acid and 2 mol/l perchloric acid, respectively. Both fractions of vanadium and cobalt are subsequently purified by anion exchange from 0.1 mol/l hydrochloric acid–3 volume% hydrogen peroxide for vanadium and 6 mol/l hydrochloric acid for cobalt. Vanadium and cobalt in the effluents are determined spectrophotometrically with 4-(2-pyridylazo) resorcinol. A 98.2% recovery of cobalt was obtained by this procedure in the presence of appreciable excesses of elements also likely to occur in the sample, namely magnesium, calcium, aluminium, iron, copper, nickel and zinc.

1.1.5 Copper

Spark source mass spectrometry, employing the stable ^{65}Cu and ^{63}Cu isotopes of copper, has been used by Harvey to study the uptake of this element [17]. An attractive feature of this method is that both the natural copper contents of the fish organs and the concentrations of added tracers are determined on the same sample by making two measurements of

Table 1.2. Copper content and accumulation of ^{65}Cu tracer in a 30 g (wet) plaice (*Pleuronectes platessa*). From [17]

Organ	Wet weight, g (A)	Percentage of body weight (B)	Total natural Cu in organ, µg (C)	Concentration of natural Cu in organ, µg/g (wet) (D)	^{65}Cu accumulated in organ after 2-month exposure at 8 µg/l (E)
Blood cells	0.15	0.5	0.04 ± 0.004	0.27 ± 0.03	0.03 ± 0.003
Blood serum	0.3	1.0	0.17 ± 0.02	0.57 ± 0.06	0.14 ± 0.01
Heart	0.02	0.06	0.06 ± 0.006	3.0 ± 0.3	0.15 ± 0.02
Spleen	0.01	0.04	0.03 ± 0.003	3.0 ± 0.3	0.13 ± 0.01
Liver	0.5	1.7	0.85 ± 0.09	1.7 ± 0.2	1.0 ± 0.1
Kidney	0.12	0.4	0.08 ± 0.008	0.67 ± 0.07	0.14 ± 0.01
Gut	0.3	1.0	0.33 ± 0.03	1.1 ± 0.1	0.25 ± 0.03
Stomach	0.15	0.5	0.12 ± 0.14	0.80 ± 0.08	0.28 ± 0.03
Gill filaments	0.2	0.6	0.12 ± 0.01	0.60 ± 0.06	0.33 ± 0.03
Skin	2.2	7.2	1.4 ± 0.1	0.64 ± 0.06	0.06 ± 0.006
Muscle	17.5	58.3	3.9 ± 0.4	0.22 ± 0.02	0.02 ± 0.002
Bone	4.0	13.2	6.4 ± 0.6	1.6 ± 0.2	0.20 ± 0.02

isotopic ratio—one before and one after the addition of the standard ^{63}Cu spike. Both of these isotopes constitute ideal tracers, since they are readily available at low cost and are free from radiation hazards. A spark source mass spectrometer is an ideal way of carrying out isotopic ratio measurements.

In this procedure, the wet fish sample was weighed before and after vacuum freeze-drying, and then transferred to a Tracerlab (Richmond, CA, USA) LTA 600 low-temperature asher to remove organic matter. The residue was then digested in 0.5 mol/l hydrochloric acid–30% hydrogen peroxide, ascorbic acid being added to destroy residual hydrogen peroxide, and copper extracted from the solution with a carbon tetrachloride solution of dithizone. This extract was then evaporated directly onto graphite prior to spark source mass spectrometric evaluation. Table 1.2 illustrates the type of data obtained in this procedure for a range of biological samples.

1.1.6 Lead

The molybdenum- and lanthanum-treated pyrolytically coated GF–AAS method described by Poldoski [15] under cadmium earlier in this section has also been applied to the determination of lead in fish tissue. Lead results obtained in spiking recovery experiments, carried out on fish tissue samples and on an authenticated reference sample (NBS SRM 1577 bovine liver) under specified conditions of analysis, were reported. It is seen that the determined lead content on NBS SRM 1577 (0.33 ± 0.01 mg/kg) is in

good agreement with the nominal value (0.34 ± 0.08 mg/kg). Average analytical recoveries on the fish samples are 91 – 93%. Down to 4 pg lead can be determined in the injected portion of sample. Lead contents obtained by this procedure are in good agreement with those obtained by anodic scanning voltammetry. Using this method, 0.26 mg/kg and 0.32 mg/kg of lead were found in whole catfish and blue gill samples, respectively.

Pagenkopf et al. [18] also employed G-AAS to determine lead in fish. They were able to determine down to 0.15 µg/kg lead in fish. In this procedure 1 – 5 g fish muscle tissue were removed by dissection and freeze-dried by a Thermovac (Copiague, NY, USA) lypholiser. Approximately 1 g of the dried tissue was weighed and then digested in a mixture of 7.00 ml of concentrated nitric acid and 5.00 ml of concentrated perchloric acid. The solutions were slowly heated until all foaming had stopped and dissolution was achieved. At this point, the temperature was increased so as to reduce the volume to about 1 ml. This was accompanied by copious fuming of perchloric acid. The maximum temperature was 88 °C. The colourless samples were then transferred to cleaned 100 ml volumetric flasks and diluted to volume. An Eppendorf pipette was used to transfer 20 µl of the sample into graphite cups. The cups were placed under an infrared light and heated until the solvent had evaporated. They were then placed in the furnace and peak absorbances were recorded. Spiking experiments in fish samples originally containing 0.12, 0.77 and 1.81 mg/kg lead indicated recoveries of 95 – 102%.

To overcome problems of contamination and nonreproducibility in the determination of low levels of lead in fish, Harms [19] devised a method of sample pretreatment and enrichment in which sample decomposition was performed in a closed system based on Mattinson's two-bottle system, and followed by the addition of pure nitric acid and then by neutralisation with ammonia and extraction with dithizone/toluene solution. After back-extraction into aqueous hydrochloric acid, the aqueous phase was subjected to measurement of the Pb-203 activity (recovery control) followed by electrothermal AAS for determination of stable lead. Samples of fish muscle containing less than 0.5 µg/kg could be analysed by this procedure.

May and Brumbaugh [20] used ammonium dihydrogen phosphate matrix modifier and a modified L'vov platform to overcome matrix interference effects in the determination of lead in fish tissues. The 283.3 lead line was used. They defined GF–AAS conditions to obtain maximum improvement in the slope ratio. Precisions were between 0.8 and 1.7% for fish samples.

Fish sample digestions were performed in PTFE-capped glass pressure reaction vessels in which the sample was digested with concentrated nitric acid at 70 °C for 48 hours. The digests were then made up to 50 ml with 1% hydrochloric acid. This procedure did not yield complete digestion, as lipids are not destroyed and remain as a floating white solid in the digest.

1.1.7 Mercury

Atomic Absorption Spectrometry

Various workers have discussed the application of cold vapour AAS to the determination of mercury in fish [21–33]. Various digestion procedures have been used, including concentrated nitric acid in a Teflon-lined sealed bomb [21] or glass tube [24], mixtures of nitric acid and sulfuric acid [29, 30], 50% hydrogen peroxide [28, 32] and sulfuric acid–potassium permanganate [27] in open tubes. Wickbold combustion procedures have also been used [33].

Hendzel and Jamieson [31] digested 0.1 – 0.5 g of fish sample with 5 ml nitric acid–sulfuric acid (1 : 1 v/v) in a glass digestion tube at 180 °C until white fumes appeared. After reduction with a reagent comprising hydroxylamine sulfate and stannic chloride, the elemental mercury was swept off with a stream of air and estimated at 253.7 nm by cold vapour AAS.

Louie [30] used concentrated hydrochloric acid–nitric acid–sulfuric acid in open tube digestions followed by cold vapour AAS to determine down to 0.01 mg/kg mercury in fish tissues. He claimed that this was an improvement over previous methods and that 3 g of fish was completely digested at 85 – 100 °C within 30 minutes. Using this procedure, Louie [30] obtained a mercury content on NBS Albacore Tuna Research Material 50 Reference Sample of 0.94 ± 0.05 mg/kg against a certified value of 0.95 ± 0.01 mg/kg. Levels found in various fish samples ranged between 0.1 and 0.4 mg/kg.

Davidson [28] used digestion on a hot plate with 4 : 1 50% sulfuric acid–hydrogen peroxide to digest tissue prior to the determination of mercury at 253.7 nm by cold vapour AAS.

An approximately 0.100 – 0.200 g portion (less if high mercury levels are known to exist) of homogenised, freeze-dried and ground tissue (or 0.500 – 1.00 g wet mass) was weighed into each reaction tube. Then 10 ml of 4+1 sulfuric acid was added and the tubes were covered and left to stand overnight. At this stage, 4 ml of cold (4 °C) 50% w/v hydrogen peroxide was mixed in and the tubes were placed on the hot block, set so that the sample temperature did not exceed 80 °C. When the solutions were clear and colourless, tubes were removed from the hot block. They were cooled in a cold water bath and 46.0 ml of cold (4 °C) 0.1% w/v potassium permanganate solution were added in a steady stream to ensure complete mixing. The required final volume was 60 ml. With argon flowing through the solution, a hydroxylamine sulfate-hydrazine sulfate–stannous chloride reductant was added and the elemental mercury swept into the AAS.

Nine replicate samples of NBS reference tuna (Research Material No. 50) were analysed by the 50% hydrogen peroxide method to determine the repeatability of the method. The mean and standard deviations were 1.00 µg and 0.02 µg dry mass, respectively, against the reported value of 0.95 ± 0.1 µg, and this indicated that 80 – 90% of the mercury content is present as

methylmercury. Between 0.24 and 1.11 mg/kg of mercury was found in pike and lake trout samples by this method.

Konishi and Takahashi [32] have described a method for the determination of inorganic mercury in fish in the presence of organic mercury. This is based on the fact that hydrogen peroxide oxidatively liberates inorganic mercury from organic substances in strong alkali, and reduces it to the metallic state without decomposing organic materials concomitantly present. The metallic mercury, vaporised with a nitrogen stream, is trapped by gold amalgamation, and then released for electrothermal atomisation AAS. The detection limit is 1 ng of inorganic mercury, and the coefficient of variation for 40 ng of inorganic mercury is 2.8%. A 92% recovery of mercury was obtained in this procedure.

Fostier et al. [40] used microwave digestion followed by automated cold vapour AAS to determine mercury in fish.

Adeloju et al. [41] evaluated four of the most commonly used wet digestion methods for mercury in fish and found that the one based on the use of a nitric–sulfuric acid mixture was the best. Subnanogram amounts of organic and inorganic mercury have been determined by helium microwave-induced plasma atomic emission spectrometry [42]. Detection limits were around 10 pg. Organic mercury was determined as the difference between total and inorganic mercury.

Liang et al. [43] carried out a simultaneous determination of monomethylmercury, inorganic mercury and total mercury using a procedure based on ethylation, room temperature precollection, gas chromatographic separation and detection by cold vapour atomic fluorescence. The detection limit was 1 pg.

Gas Chromatography

Jones and Nickless [34] converted inorganic mercury in fish samples to its methyl derivative using 2,2'-dimethyl-2-silapentane-5-sulfonate as a reagent, prior to the determination of inorganic mercury in benzene extracts of the reaction product by gas chromatography. The highest yield was obtained by digesting the fish sample at 100 °C with 5 N nitric acid in the presence of sodium nitrite and then extracting with benzene. Between 2.8 and 8.6 mg/kg mercury were found in fish samples by this method.

Pyrolysis Ultraviolet Spectroscopy

Thomas et al. [35] described a rapid pyrolytic procedure for determining the total mercury content in fish. A weighed amount of homogenised fish tissue is combusted in a flowing air stream at 900 °C, and then over copper oxide at 850 °C to ensure complete combustion. Elemental mercury vapour is expelled into the carrier stream and, after passing through silver oxide absorbent traps to remove possible interfering gases, is detected and measured in an ultraviolet photometer at 253.5 nm. The relative error is approximately

Table 1.3. Comparison of mercury analysis in various fish specimens. From [35]

Fish	Combustion method total Hg, mg/kg	Digestion method total Hg, mg/kg	Mercury as methyl Hg, mg/kg
Carp	2.7	1.5	2.4
		1.6	2.3
Shiner	0.39	0.33	0.33
		0.28	0.35
Chub	0.19	0.10	0.16
		0.09	0.10
Buffalo	0.33	0.14	0.41
	0.53	0.12	
	0.44		
Carp	0.54	0.28	0.47
	0.64	0.29	
	0.52		
Blue Cat	0.25	0.21	0.21
	0.26	0.27	
Channel Cat	0.52	0.37	0.42
	0.47	0.55	
Carp	0.25	0.31	0.26
		0.34	0.22
Crappie	0.14	0.12	0.09
		0.12	0.11
Crappie	0.20	0.19	0.13
		0.14	0.11

$\pm$10% for inorganic and organic mercury over a linear response range of 0.05 to 3.0 mg/kg.

Thomas et al. [35] compared mercury contents obtained by this method with those based on a gas chromatographic method involving the conversion of inorganic mercury to methylmercury, and with determinations of total mercury by a sulfuric acid–potassium permanganate acid digestion method.

It is seen in Table 1.3 that, whereas total mercury determinations are lower, the pyrolysis method and the gas chromatographic methods give results that are in reasonably good agreement.

Anodic Stripping Voltammetry

Nitric acid–perchloric acid digestion in a Teflon autoclave bomb has been used to prepare digests of finely powdered freeze-dried fish [36]. The extract was irradiated with ultraviolet light to complete fish sample degradation prior to the determination of mercury using a gold disc electrode. Results obtained compared well with total mercury contents obtained by neutron activation analysis.

Neutron Activation Analysis

Uthe et al. [22] found that mercury determinations in fish by digestion–flameless AAS were only slightly lower than those obtained by neutron activation analysis, but had a poorer precision.

Sivasankara-Pillay et al. [37] determined mercury in fish samples by neutron activation analysis. As a further check, the samples were wet-ashed at 120 – 160 °C with sulfuric and perchloric acids in the presence of an accurately known amount of mercury carrier. A preliminary precipitation as mercury sulfide is followed by further purification, and electrodeposition or precipitation as mercuric oxide to isolate mercury. The radioactivities due to ^{196}Hg and ^{197}Hg are then measured by scintillation. The errors in this method are 5% at the 2 mg/kg mercury level and 15% at the 0.01 mg/kg level, with standard deviations of less than 5% at the 5 mg/kg level and less than 17% at the 0.01 mg/kg level, respectively. Fish samples contain both organic and inorganic mercury, predominantly organic. Sivasankara-Pillay [37] showed that freeze-drying of homogenised fish samples caused a 16 – 39% loss of organic mercury compounds, but did not cause any loss of inorganic mercury. Similarly, low-temperature ashing (Tracerlab Model 505 asher) caused an 81 – 98% loss of mercury from fish. Exposure of fish samples to X-rays or neutrons before mercury analysis, in order to convert volatile organomercury compounds to inorganic mercury, reduced mercury losses to 4.5 – 16.4% but did not eliminate them. Low-temperature (60 °C) oven drying caused up to 72% losses of volatile mercury from fish. As a consequence of these findings, Sivasankara-Pillay et al. [29] decided that it was good practice before analysis not to preprocess fish samples to limit their bulk or to reduce their water content, and not to store samples in containers that adsorb mercury onto their surfaces. The procedure they adopted was to keep the samples frozen until use. They were then homogenised using a blender and/or a grinder made of stainless steel or borosilicate glass. The portion of sample used for neutron activation analysis was then vacuum-sealed in a polyethylene bag.

Table 1.4 shows the mercury contents obtained by neutron activation analysis in a survey of fish in Lake Erie.

Table 1.5 presents the results obtained in an interlaboratory comparison of methods for the determination of naturally occurring forms of mercury in fish. It is seen that, in general, the highest results are obtained by neutron activation analysis.

Lo et al. [38] digested wet fish samples with concentrated sulfuric–nitric acids until white fumes appeared, and then added excess potassium permanganate, sodium chloride and hydroxylamine hydrochloride to reduce mercury.

Mercury in the digest was then preconcentrated into a small volume of lead diethyldithiocarbamate dissolved in chloroform. The chloroform was then allowed to evaporate in an ampoule, and the ampoule sealed for neutron activation analysis and subsequent gamma spectrometry of the sensitive

Table 1.4. Mercury content of edible tissues of Lake Erie fish (1970 fall catch). From [37]

Species	Mercury content of edible tissue mg/kg		
	Western basin	Central basin	Eastern basin
Walleye	0.79 (25)[a]	0.65 (25)	0.33 (25)
Yellow perch	0.61 (25)	0.49 (25)	0.29 (25)
White bass	0.60 (25)	0.72 (25)	0.43 (25)
Channel fish	0.36 (25)	0.42 (20)	–
Freshwater drum	0.67 (25)	0.62 (20)	0.30 (25)
Carp	0.23 (25)	0.35 (17)	0.36 (14)
Coho salmon	0.69 (20)	0.58 (10)	0.51 (13)
White sucker	0.55 (24)	0.56 (8)	0.35 (25)
Gizzard shad	0.20 (25)	0.21 (15)	0.26 (18)
Smallmouth bass	–	0.55 (14)	–
Smelt[b]	–	–	0.30 (10)

[a] The numbers in the brackets refer to the number of fish samples of a particular species used when preparing the composite.
[b] Mercury content of the whole fish.

Table 1.5. Results of mercury analysis method evaluation program using fish homogenates[a]. From [37]

Analytical method used	Number of labs that participated	Range of reported values in ppm Hg		
		Sample D	Sample E	Sample G
Flameless (cold) atomic absorption	13	0.09 to 1.80	0.03 to 0.18	2.80 to 5.21
Flame atomic absorption	5	0.70 to 1.80	< 0.05 to 0.49	2.26 to 5.40
Dithizone colorimetry	1	1.31	0.05	3.98
Dithizone titration	1	0.09	< 0.03	0.09
Pyrolysis	2	0.47 to 1.52	0.04 to 0.10	2.00 to 4.25
Neutron activation analysis	6	0.95 to 1.77	0.04 to 0.19	2.83 to 4.60
Cold atomic absorption following acid digestion (Fresh Water Institute, Winnipeg, Canada).		1.46	0.04	4.53
Neutron activation analysis with post-irradiation chemical separation (Western New York Nuclear Research Center).		1.77	0.12	4.56

[a] Trace Mercury Analyses Evaluation Program sponsored by the Fresh Water Institute of the Canadian Fisheries Research Board

^{197}Hg peak. As well as reducing the detection limit to 1 µg/kg in fish, preconcentration has the additional advantage of overcoming interferences from ^{24}Na and ^{82}Br which commonly occur in fish samples. Recoveries of 95% of mercury in fish samples were obtained by this procedure.

Medina et al. [39] has described a high-performance liquid chromatographic method for the determination of mercury speciation in fish.

1.1.8 Nickel

Pihlar et al. [44] have described a voltammetric procedure for the determination of µg/kg (1 ng/l) levels of nickel in various biological materials including fish. The sample is wet-digested with nitric acid (65%) sulfuric acid (98%) in the ratio 5 : 1 or 2.5 : 1 at 150 – 200 °C. Alternatively, a 30-minute digestion with 30% hydrogen peroxide is carried out. The digest is then buffered at pH 9.2 using 0.1 – 1 M ammonia/ammonium chloride. The dimethylglyoxime complex is then formed and dc or differential pulse voltammetry at −1.25 V is applied to determine the nickel. The method gave 4.6 µg/kg nickel in a fish sample.

1.1.9 Selenium

The semi-automated atomic absorption method [4] for the determination of arsenic in fish described in Sect. 1.1.2 has also been applied to the determination of selenium. Average recoveries of selenium(IV), selenium(VI), selenourea, selenomethonine and selenocysteine obtained by this method were between 91 ± 10 and 100 ± 1%. Selenium found in a NBS standard bovine liver (SRM 1577) reference sample was 1106 ± 100 mg/kg against a certified value of 1020 ± 40 mg/kg. Selenium levels found in a range of fish samples ranged from 0.308 to 0.548 mg/kg, determined with a coefficient of variation of 4.5 to 6.0%.

The semi-automated inductively coupled plasma atomic spectrometric technique described by Goulden et al. [6] in Sect. 1.1.2 has also been applied to the determination of selenium in nitric–sulfuric–perchloric acid digests of fish.

Januzzi et al. [45] have reported a method for the determination of selenium in fish based on a slurry technique without sample preconcentration.

1.1.10 Strontium

Bagenal et al. [46] have described a method for the determination of µg/g levels of strontium in fish scales. The sample is digested with perchloric acid and the flask heated to destroy organic matter. A nitric acid solution

of this digest is used for the determination of strontium by GF–AAS. In general, freshwater trout were found to contain between 76 and 142 µg/kg strontium in their scales, whilst sea trout had much higher levels present in their scales (320 – 653 µg/kg).

1.1.11 Tin

Flameless AAS [47] has been applied to the determination of tin in fish. Between 0.4 and 6.6 mg tin was reported in homogenised fish samples. Sample digestion was carried out using lumatron (a quaternary ammonium hydroxide) dissolved in isopropanol (available from H. Kurenell, Neuberg, Germany).

1.1.12 Vanadium

The combined ion-exchange spectrophotometric procedure [16] described in Sect. 1.1.4 on cobalt earlier in this chapter has been applied to the determination of vanadium in cutlass fish. A recovery of 96.3% vanadium was obtained by this procedure.

Cation exchange chromatography followed by neutron activation analysis has been used [48] to determine down to 30 µg/kg vanadium in fish.

1.1.13 Multi-cation Analysis

1.1.13.1 Atomic Absorption Spectrometry

Various workers have discussed the application of this technique to the determination of elements in fish tissue digests [49–53]. Elements determined include cadmium, lead, copper, manganese, zinc, chromium and mercury [54]; cadmium, zinc, lead, copper, nickel, cobalt and silver [50]; copper, zinc, cadmium, nickel and lead [52]; lead, cadmium, copper and zinc [52]; and lead and cadmium [53].

Various digestion systems have been studied for the decomposition of fish samples prior to analysis, including digestion with nitric acid–perchloric acid [50, 52], nitric acid–hydrogen peroxide [51, 53], all in open tubes, or decomposition with nitric acid in a closed Teflon-lined bomb [49].

Nitric Acid–Sulfuric Acid Digestion

Agemian et al. [52] have reported a simple and rapid digestion method for the simultaneous acid extraction of chromium, copper, zinc, cadmium,

nickel and lead from high-fat fish tissue. Samples are digested with nitric (5 ml 16N) and sulfuric (5 ml 36N) acids at 150 °C in a modified aluminium hot-block. The method is specially set-up for fish sample sizes of up to 5 g for low-level detection of these elements. After digestion, acid extracts of the sample are analysed by direct flame AAS for copper, zinc and chromium. The other three elements, cadmium, nickel and lead, are concentrated by chelation with ammonium tetramethylene dithiocarbamate followed by solvent extraction with isobutyl methyl ketone, and determined by flame AAS.

Detection limits in whole fish tissue are 0.02 mg/kg (cadmium), 0.05 mg/kg (nickel), 0.1 mg/kg (lead) and 0.2 mg/kg (chromium, copper and zinc). Recoveries through the whole analytical procedure ranged from 90 to 110%. Precisions were in the range 9.1% to 12.1 (cadmium), 5 – 15% (nickel and copper), 4.3 – 17.0% (lead), 3.9 – 6.7% (zinc) and 7.9 – 15% (chromium).

Nitric Acid–Perchloric Acid Digestions [50]

To carry out this digestion, 0.5 – 3 g of ball mill-ground freeze-dried fish sample is digested in a silica flask with 10 – 20 ml concentrated nitric acid and then 5 – 10 ml of 1 : 1 nitric : perchloric acid to dryness. The residue is dissolved in dilute hydrochloric acid–nitric acid and adjusted to pH 8 with ammonia. This solution is extracted with a 0.02% solution of dithizone in chloroform. Metals are then back-extracted from the organic phase with 2 mol/l hydrochloric acid prior to atomic absorption spectrometry. Using this method, the following values (mg/kg) were obtained for a NBS reference kale sample (nominal values in brackets): cadmium 0.9 (0.84); zinc 29.9 (31.8); lead 2.6 (3.2); copper 4.2 (4.9); cobalt 0.05 (0.056). Concentrations (mg/kg) of metals found in whale tissues were: zinc 26 – 103; lead 0.45 – 1.37; copper 1.2 – 7.6; nickel 0.17 – 0.60; cobalt 0.07 – 0.38; silver 0.02 – 0.04; cadmium, not detected. Kale brought from Iceland contained the following concentrations: zinc 39; lead 0.89; copper 2.6; nickel 0.34; cobalt 0.14; silver 0.04 mg/kg; cadmium, not detected.

Nitric Acid–Hydrogen Peroxide Digestions

Van Hoof and Van San [51] worked on fish samples that had been calcined at 450 °C prior to digesting the ash in 2.5 : 1 v/v 14 N nitric acid: 30% hydrogen peroxide. Elements determined included copper, zinc, cadmium and chromium. Low recoveries of at least some of these elements would be expected under these conditions.

Borg et al. [53] digested 10 mg freeze-dried fish livers with concentrated nitric acid at 50 °C for 2 hours in quartz tubes, and then slowly raised the temperature to 110 °C over 18 hours. Hydrogen peroxide (30%) is added to the cooled samples, which are again heated to 110 °C for six hours to digest fats completely. When made-up to a standard volume, this digest was used for the determination of copper, lead, cadmium and zinc by GF–AAS. Table 1.6 compares results for fish livers obtained by this procedure with

those obtained via neutron activation analysis. The high metal concentrations found in the livers reflect the fact that the fish were taken in an area subject to heavy contamination originating from ore smelting activities.

Nitric Acid Bomb Digestion

Ramelow et al. [49] determined cadmium, lead, copper, manganese, zinc and chromium in wet fish by digesting a 0.5 – 1.0 g sample with 2 – 3 ml concentrated nitric acid in a Teflon-lined bomb at 150 °C for 1.5 hours. Elements

Table 1.6. Metal concentrations in fish liver determined by the Borg method (AAS) and by neutron activation analysis (NAA). From [53]

	mg/kg					
	Zn		Cu		Cd	
Sample No.	AAS	NAA	AAS	NAA	AAS	NAA
Perch						
173	120	131	13	17.80	5.1	4.79
174	120	119	12	11.70	3.8	4.31
178	100	107	11	10.70	2.0	2.45
189	120	130	6.7	8.64	6.9	8.08
191	100	115	7.2	8.30	2.8	3.30
358	150	115	6.0	8.38	8.1	7.46
361	110	112	5.7	9.96	5.2	6.73
364	120	124	5.3	8.21	6.8	9.01
368	120	107	3.7	5.69	6.2	7.34
236			22	23.9	2.1	2.1
244			23	21.8	1.5	1.6
249			55	45.0	2.2	2.6
264			48	46.2	4.0	4.0
White fish						
463			27	24.5	0.56	0.51
477			62	56.0	0.90	0.84
482			–	–	0.72	0.71
487			43	39.6	0.19	0.275
Pike			11.7 ± 0.6 ($n = 3$)	10.0	0.17 ± 0.02 ($n = 3$)	0.162

Table 1.7. Analytical results from the analysis of trace metals in various marine organisms (results show mg/kg fresh weight). From [49]

Species	Cd	Pb	Cu	Mn	Zn	Cr
White bream	0.04	0.61	1.11	0.51	10.6	0.58
Sardine	0.02	0.57	2.18	1.63	6.3	0.28
Gilt-head bream	0.03	0.68	1.20	–	9.5	0.49
Grey mullet	0.09	1.36	1.70	0.33	12.2	0.10
Horse mackerel	0.17	1.05	0.99	0.63	4.3	0.65
Striped mullet	0.02	0.12	0.68	0.22	6.4	0.14

were determined in the digest by flame atomisation or graphite furnace atomisation AAS. Concentrations found in whole fish in an unpolluted area are shown in Table 1.7, which should be contrasted with concentrations found in fish livers in a polluted area (Table 1.6).

Comparison of Digestion Methods

Adeloju et al. [54] evaluated four commonly used wet digestion procedures for fish and found that a method based on digestion with a mixture of nitric and sulfuric acids gave the best results.

Intercomparison Studies

The International Council for the Exploration of the Sea has arranged a series of intercomparison studies of the determination of trace elements in fish using techniques based on AAS. A summary of the test results is given in Table 1.8.

Despite the large number of participants in the fourth exercise, the results for the analysis of copper, zinc and mercury demonstrated that most analysts were continuing to produce reasonably comparable and accurate data for these metals at levels typical of those found in fish muscle and shellfish tissue. The results for mercury were particularly good in view of the relatively low concentrations present.

The analysis of arsenic appears to have posed problems for some of the analysts in view of the wide range of values reported in the fourth exercise, i.e., 6.27 – 275 µmol/kg. An independent check of arsenic in the sample by neutron activation analysis produced a mean value of 200 µmol/kg with a coefficient of variation of 6%. With the exception of one analyst, who used x-ray fluorescence (mean arsenic concentration of 216 (µmol/kg), all analysts employed a similar, but individually modified, procedure for the analysis of arsenic: following destruction of the organic matter by wet digestion or dry ashing, the arsenic was liberated from the resultant matrix as arsine and then measured by either flame and flameless AAS or colorimetry. If it is assumed that the results produced by x-ray fluorescence and neutron activation analysis represent the true concentration of arsenic in the reference material, then the low results produced by some participants are incorrect. It follows that the methods used by these analysts may suffer from some form of matrix interference.

From an analysis of the arsenic methodology, it appears that the root of the analytical problem may lie with the choice of technique used for the destruction of organic matter. This is suggested by the fact that all methods incorporating a dry ashing step produced high values (> 133 µmol/kg), whereas some methods employing a wet digestion step produced very low values, in the range 6.7 – 119 µmol/kg. Some of the wet digestion procedures which produced high values appear to have overcome the effects of matrix interference through either the addition of nickel salts to the digest before