

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 $\mu\text{g/kg}$ strontium in their scales, whilst sea trout had much higher levels present in their scales (320 – 653 $\mu\text{g/kg}$).

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 $\mu\text{g/kg}$ 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* 14N 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]

Sample No.	mg/kg					
	Zn		Cu		Cd	
	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 $\mu\text{mol/kg}$. An independent check of arsenic in the sample by neutron activation analysis produced a mean value of 200 $\mu\text{mol/kg}$ with a coefficient of variation of 6%. With the exception of one analyst, who used x-ray fluorescence (mean arsenic concentration of 216 ($\mu\text{mol/kg}$), 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 ($> 133 \mu\text{mol/kg}$), whereas some methods employing a wet digestion step produced very low values, in the range 6.7 – 119 $\mu\text{mol/kg}$. 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

Table 1.8. Summary of the results from the analysis of reference materials distributed in the ICES metals intercomparison exercise (1971 – 1980) (from author's own files)

Elements	Exercise	No. of participants	Range of mean values submitted, $\mu\text{mol/kg}$	Grand mean, $\mu\text{mol/kg}$	SD	CV	Outliers (or qualifications)
Copper	4a	36	< 6 – 63	28	11	39	< 6 as 6
Zinc	4a	36	199 – 566	352	61	18	None
Mercury	4a	33	0.25 – 1.9	1.05	0.35	33	None
	4b	34	< 0.05 – 1.3	0.3	0.15	50	All < values (two) and two high values (1.05 and 1.25) omitted
Cadmium	4a	35	0.05 – 8.8	0.29	0.24	87	All < values (five) and four high values (2.5, 2.8, 3.5 and 8.8) omitted
Lead	5a	52	4.7 – 9.9	7.1	4.8	17	None
	4a	32	0.96 – 36.0	1.0	0.7	71	
	5b	52	1.06 – 37.4	13.0	6.1	47	Two high values (29.3 and 37.4) omitted
	5c	32	0.53 – 15.4	3.6	2.5	71	One high value (15.4) omitted
Arsenic	4a	16	6.7 – 275	196	56	28	Three low values (6.7, 8.4 and 21.3) omitted
Exercise	(Year)	Raw Material	Brief description of preparation of reference material			Elements under study	
4a	(1978)	Fish fillet (cod, skinned)	Wet tissue cut into small pieces (3 cm \times 3 cm); blast-frozen, freeze-dried and repeatedly ground in a hammer mill to a fine flour			Cu, Zn, Hg, Cd, Pb and As	
4b		Fish fillet (cod, skinned)	Chopped wet tissue washed with dilute acid to reduce Hg content. Freeze-dried and ground into flour as above			Hg only	
5a	(1980)	White meat of edible crab	As for 4a			Cd only	
5b		Commercial fish meat	As for 4a			Pb only	
5c		Hepato-pancrease of lobster	Prepared in the form of acetone powder			Pb only	

SD: Interlaboratory standard deviation

CV: Interlaboratory coefficient of variation

measurement by flameless AAS or the utilisation of a much stronger reducing agent at the arsine generation stage. It appears that some component(s) of the matrix, which is destroyed or eliminated during dry ashing but not during wet digestion, suppresses the release of arsenic as arsine and also suppresses the arsenic signal in flame and flameless AAS unless nickel salts are added to the digest prior to measurement.

The results from the fifth exercise show that the majority of participants can produce comparable (i.e., interlaboratory coefficient of variation of 10%) and accurate data for cadmium but not for lead.

In conclusion, over the nine years of the intercomparison exercise, a progressive improvement was shown in the determination of copper, zinc and mercury. Difficulties were still being encountered in relation to producing comparable data for lead and cadmium at low tissue concentrations in the range 0.05–0.9 µmol/kg, but at higher tissue concentrations (2–12 µmol/kg) there have been few problems producing accurate data for cadmium, although somewhat greater difficulties in the case of lead.

Ramelow et al. [49] digested fish samples with concentrated nitric acid in a Teflon-lined bomb for 1.5 hours at 150 °C prior to the determination of mercury by reduction to elemental mercury with stannous chloride and determination by cold vapour AAS.

1.1.13.2

Hydride Generation Atomic Absorption Spectrometry (HG-AAS)

Welz and Melcher [55] decomposed fish tissue with nitric-sulfuric-perchloric acids in a Teflon-lined bomb to decompose arsenic, selenium and mercury. Nitric acid alone gave low recoveries for arsenic and selenium but quantitative recovery for mercury. The final determination of down to 0.3 mg/kg arsenic, 0.2 mg/kg selenium and 0.005 mg/kg mercury was carried out by hydride generation and cold vapour AAS.

1.1.13.3

Isotope Dilution Coupled Plasma Mass Spectrometry (IDC-PMS)

Buckley and Ihnat [56] determined trace elements in fish samples by isotope dilution ICP-MS.

1.1.13.4

Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)

The ICPES [57] procedure has been applied to the determination of arsenic, antimony and selenium in fish. Sample digestion was carried out in open vessels at room temperature using nitric acid, followed by heating with a mixture of nitric, perchloric and sulfuric acids on a hot plate. Accurate determinations (mg/kg) were obtained by this procedure. On NBS Reference Sample NBS 1566 (oyster tissue), for arsenic the method gave (certified

values in brackets) 11.1 ± 1.1 (13.4 ± 0.9), antimony 0.42 ± 0.3 , and selenium 1.7 ± 0.2 S (2.1 ± 0.5) and for NBS 1571 (orchard leaves) it gave for arsenic 11.9 ± 0.6 (10.2 ± 2.0), and antimony 2.8 ± 0.02 (0.08 ± 0.01).

Sakai and May [58] used ICPAES, AAS and hydride generation AAS to determine cadmium, arsenic, boron, chromium, mercury, molybdenum, nickel, lead and selenium in common carp. The highest concentrations found were: arsenic 1.5 mg/kg, boron 20 mg/kg, cadmium 0.27 mg/kg, chromium 2.2 mg/kg, mercury 2.9 mg/kg, molybdenum 3.6 mg/kg, nickel 2.2 mg/kg, lead 2.3 mg/kg and selenium 5.5 mg/kg.

1.1.13.5

Differential Pulse Anodic Stripping Voltammetry (DPASV)

Adeloju et al. [59] used this technique to determine selenium, copper, lead and cadmium in fish tissues. Detection limits were in the $\mu\text{g/kg}$ range. Samples were first digested with concentrated nitric acid and 80% magnesium nitrate solution, and then dry ashed at 500°C . The ash was dissolved in boiling 6 mol/l hydrochloric acid. This solution was analysed for selenium on a hanging mercury drop polarographic analyser, and copper, lead and cadmium were determined in the anodic scanning voltammetry mode using the peaks appearing at -0.20 , -0.5 and -0.7 V versus SCE, respectively. Results (mg/kg) obtained by this method for crayfish are in good agreement with certified values (reference values in brackets): selenium 0.17 (0.16), copper 3.46 (3.10), lead 0.48 (0.48) and cadmium 0.10 (0.05). Relative standard deviations in determinations of selenium, copper, lead and cadmium were 12, 5, 15 and 20%, respectively.

1.1.13.6

Neutron Activation Analysis (NAA)

This technique has been applied to the determination of cobalt, chromium, selenium, silver, rubidium, nickel and zinc [60], and aluminium, gold, bromine, calcium, chlorine, cobalt, chromium, copper, iron, iodine, potassium, magnesium, manganese, sodium, rubidium, scandium, vanadium and tungsten [61] in fish.

Neutron activation analysis has been used to determine miscellaneous elements in fish at sub-ng/g concentrations [62].

1.1.13.7

Secondary Ion Mass Spectrometry and X-Ray Spectrometry (SIMS/XS)

This technique can be used [63] to provide simultaneous morphological and chemical identifications in histological sections of fish, molluscs and crustaceans.

1.1.13.8

Miscellaneous

Topping (private communication) has reviewed methodology for the determination of copper, zinc, mercury, cadmium and lead in fish flesh, fish flour and shellfish, and has organised intercomparison tests. Various techniques were applied, including neutron activation analysis, x-ray fluorescence and AAS.

Throughout this study, the participants, particularly those who took part in all of the exercises, showed a progressive improvement in the analysis of copper, zinc and mercury. On the basis of these results, it is concluded that the analytical data produced by these participants for these metals in a fish and shellfish monitoring programme are comparable.

The identification of significant differences in the concentrations of working standards and the subsequent adoption of a common procedure for the preparation of these solutions are considered to be important factors in the achievement of this improved comparability for the above metals.

The study revealed that the participants were unable to produce comparable, and in most cases accurate, data for lead and cadmium at low tissue concentrations, i.e., in the range 0.024–4.8 $\mu\text{mol/kg}$ and 0.009–0.89 $\mu\text{mol/kg}$, respectively. However, at relatively high tissue concentrations (2.5–12.0 $\mu\text{mol/kg}$ and 10 pmol/kg , respectively), the majority of analysts experienced little difficulty in producing accurate data for cadmium, but the analysis of lead presented some problems for a minority of the participants. On the basis of these results, it is considered that the participants in ICES fish and shellfish monitoring programmes can produce comparable data for cadmium in shellfish tissue but not for cadmium in fish muscle or lead in both fish muscle and shellfish tissue.

Das [64] has reviewed the trace metal status of methods used in marine biological samples.

Arslan et al. [65] have shown that Toyo Peasil AF-Chelate 650M gives a 25-fold preconcentration in the determination of metals in juvenile bluefin tuna fish in the Pacific Ocean.

1.2

Organic Compounds

1.2.1

Hydrocarbons

Farrington et al. [66] used column chromatography and thin-layer chromatography to isolate hydrocarbons (arising from marine contamination) in fish lipids. The hydrocarbon extracts were then examined to select those that could be determined by gas chromatography mass spectrometry, by combinations of spectrophotometric methods, or by wet chemistry. As a

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