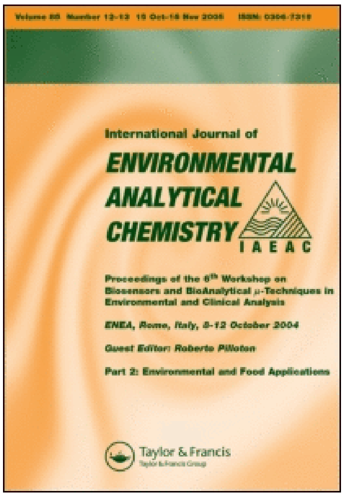

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S. E. Kakulu[a][b]; O. Osibanjo[a]; S. O. Ajayi[a]
[a] Department of Chemistry, University of Madan, Nigeria [b] Abubakar Tafawa Balewa College, Ahmadu Bello University, Bauchi, Nigeria

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Comparison of Digestion Methods for Trace Metal Determination in Fish

S. E. KAKULU,* O. OSIBANJO† and S. O. AJAYI
Department of Chemistry, University of Ibadan, Nigeria

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The analytical comparison of some digestion methods used for trace metals determination in fish by flame atomic absorption spectrophotometry (AAS) was studied. The results were also compared with those obtained for metals analysis by inductively coupled argon plasma-atomic emission spectrometric technique (ICP–AES). Both techniques showed good correlation. The average metal levels found in the fishes and shellfishes analysed from the study area by AAS technique were much lower than the World Health Organisation's acceptable limits for metals in foods.

KEY WORDS: Digestion methods, trace metal determination, fish and shellfish, atomic absorption spectrophotometry, inductively coupled-argon plasma.

INTRODUCTION

In order to evaluate the concentration and effect of trace heavy metals in the environment, analysis of biological organisms, for instance fish,[1–3] have been a valuable source of this information. The difficulties associated with the determination of trace heavy metals in biological organisms are not associated principally with the

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*Present Address: Abubakar Tafawa Balewa College, Ahmadu Bello University, Bauchi, Nigeria.

†To whom all correspondence should be addressed.

reliability of the instrumental techniques, but with the choice of sample preparation methods. The methods of sample preparation[4–9] in use involve the destruction of the organic matter either by the use of oxidizing acid(s) singly or as a mixture; or in the dry state with atmospheric oxygen. The wet-ashing procedures described for the determination of trace metals in biological samples include: $HNO_3+HC10_4+H_2SO_4$ and $HNO_3+H_2SO_4^4$, $HNO_3+H_2SO_4$ with V_2O_5 as catalyst for Hg, Cu and Zn determination[5] and $HNO_3+H_2O_2$ (30%) for the determination of Hg, Cd, Cu, Pb and Zn.[6,7]

The dry ashing procedure is usually at a temperature of about 500°C with or without ashing aids.[5,8,9]

This study compared three sample preparation procedures, namely: wet digestion using $HNO_3—H_2SO_4$ and $HNO_3—H_2O_2$ and dry ashing without ashing aid for the determination of Pb, Cu, Ni, Zn, Mn and Fe contents of fish and shellfish samples from the Niger delta area of Nigeria with flame atomic absorption spectrophotometry (FAAS) and inductively coupled argon plasma atomic emission spectrometric (ICP–AES) techniques.

EXPERIMENTAL

1. Reagents

Standard stock solutions of metals (1,000 μg per ml) were prepared from either the metal or a soluble salt of the metal of highest purity (analytical grade reagent) available.

Copper and zinc stock solutions were prepared from their metals by dissolving with concentrated nitric acid and hydrochloric acid respectively. Lead was prepared from its nitrate. Nickel and manganese were prepared from their acetate salts. Iron was prepared from iron (II) sulphate to which sulphuric acid was added. With the exception of zinc and iron which stock solutions were about 2% (V/V) hydrochloric acid and sulphuric acid concentrated respectively all other metals solution were made up to mark with 2% (V/V) HNO_3 solution.

All other reagents were analytical grade available commercially.

2. Sample collection and preparation

Most of the fish and shellfishes analysed were economic species and were purchased from artisanal fishermen in different locations in the Niger delta area of Nigeria[10] (Figure 1). All the fish and shellfish samples were washed with distilled water and then frozen till analysis.

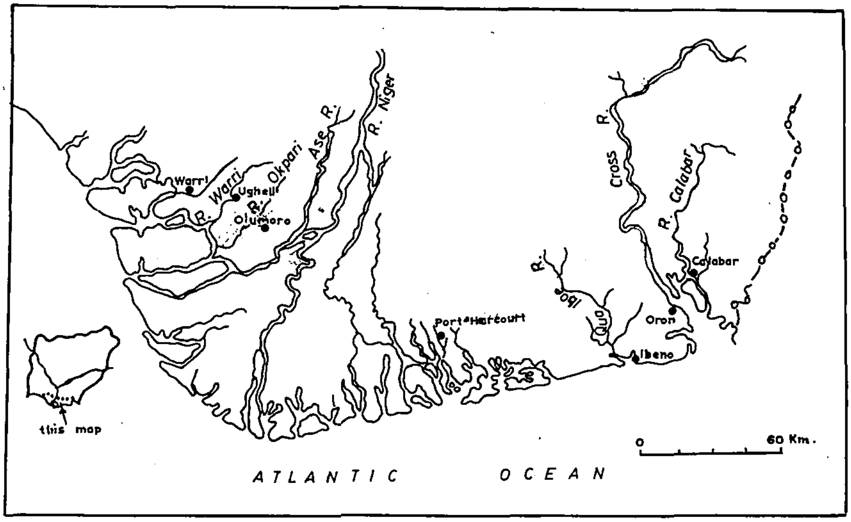

Figure 1 Niger Delta sample locations.

During sample analysis, the frozen samples were allowed to thaw. For each fish sample, the lateral muscles representing the edible portion of the fish were removed with clean plastic knife and homogenised by cutting to pieces and mixing well in a pyrex glass mortar until a homogeneous mass was obtained. For shellfishes, abdominal muscles were removed with a plastic knife from all the specimens belonging to the same sample species and pooled together in a pyrex glass mortar to obtain composite samples which were then analysed.

The efficacy of the sample homogenisation method was estimated by analysing 5 replicate samples of a homogenate for trace metals after acid digestion. The coefficient of variance for the analysis of the 5 replicates was less than 20%.[11]

3. Digestion procedures

1) *Nitric acid–sulphuric acid system*: The Analytical Methods Committee[12] method utilizing a modified Bethge apparatus[13] was used. 10.0 g of fresh weight of sample was placed in the round bottom flask and 10.0 ml of nitric acid–sulphuric acid mixture was added. This was refluxed gently until the initial reaction subsided, after which a strong refluxing for about 4 h was carried out during which complete oxidation would have occurred leading to a slightly yellow liquid in the flask. The contents of the flask and condenser were cooled and rinsed with distilled water into a 25 ml volumetric flask and finally diluted to volume with distilled water.

2) *Nitric acid–hydrogen peroxide system*: The FAO[7] method involving the digestion of the sample in an open beaker on a hot plate was used. 10.0 g of fresh weight of samples were placed in a beaker and 15 ml of freshly prepared 1:1 (V/V) nitric acid–hydrogen peroxide was added. The beaker was covered with a watchglass and set aside for about an hour in order to allow the initial reaction to subside. The beaker and its contents were heated on a hot plate, with the temperature of the hot plate not exceeding 160°C. Boiling was continued for about 2 h and subsequently reducing the volume to between 2 and 5 ml. The contents of the beaker were transferred into a 25 ml volumetric flask and diluted to volume with distilled water.

3) *Dry ashing method*: 12.0 g fresh weight of each sample in a vitreosil crucible were dried in an oven at 105°C overnight. These were transferred into a cold muffle furnace. This was set at 200°C for 1 h, after which the temperature was increased to 300°C at 50°/h. The temperature of the furnace was finally increased to 500°C after about 2 h at 300°C at a rate of 50°/h and left at this temperature for 16 h.[5,9] The residues were allowed to cool to room temperature followed by the dissolution of the ash with 3 ml of 6 M HNO_3. Dissolution was aided by warming samples on a hot plate. Sample solutions were quantitatively transferred into 25 ml volumetric flasks and diluted to volume with distilled water.

In all the procedures employed, blanks were prepared to determine the effect of the reagents' purity on the metal levels determined. The precision and accuracy of each digestion method was ascertained by analysing 12 replicates of each sample followed by the analyses of homogenate samples spiked with known metals concentration which have also been taken through the work-up procedures.

4. Instrumentation

Total metal measurements were performed by a Perkin Elmer atomic absorption spectrophotometer model 460 with air-acetylene flame, coupled to a Perkin Elmer 56 recorder and using the appropriate hollow cathode lamps. The metals were also determined using an inductively coupled argon plasma optical emission spectrophotometer (Jarrel–Ash) model 975 plasma Atomcomp with a Texas Instruments incorporated silent 700SR electronic data terminal.

RESULTS AND DISCUSSION

Table I shows the results obtained using the various digestion methods for trace metal determination in fish and shellfish samples by flame atomic absorption spectrophotometry. With the exception of lead, there was no significant difference in the concentrations of other metals using all three digestion methods as shown statistically by the *t*-test. There were significant differences for lead using the HNO_3—H_2SO_4 procedures compared with the HNO_3—H_2O_2 or the dry ashing procedures. This could be due to the loss of lead by precipitation of the insoluble lead sulphate.[4] This loss was also reflected in the recovery studies which was only 53% compared with an average of 88% for the other methods. The percentage recoveries for other metals varied from 90–94% for Cu, 88–96% for Zn, 85–90% for Ni, 93–102% for Mn and 94–98% for Fe using all three procedures.

As a result of the loss of lead in the HNO_3—H_2SO_4 procedure, the long time necessary to effect digestion of samples by dry ashing and the good recoveries obtained for all metals by the HNO_3—H_2O_2 procedure (Table I), the latter procedure was therefore used in the preparation of fish and shellfish samples prior to the determination of trace metals by atomic absorption spectrophotometry. Since systematic errors are associated with every analytical technique used

Table I Comparative efficiency of various digestion methods in the determination of trace metals in fish samples (μg g^{-1} wet weight) by atomic absorption spectrophotometry

	HNO_3—H_2SO_4	HNO_3—H_2O_2	Dry ashing
Copper			
Average	0.89	0.87	0.83
Standard deviation	0.07	0.07	0.09
Average recovery, %	94	92	90
Coeff. var., %	8	8	11
Zinc			
Average	19.84	20.86	19.56
Standard deviation	3.14	2.57	2.30
Average recovery, %	88	94	96
Coeff. var., %	16	12	12
Lead			
Average	0.35	0.68	0.77
Standard deviation	0.13	0.08	0.10
Average recovery, %	53	88	88
Coeff. var., %	37	12	13
Nickel			
Average	0.51	0.44	0.47
Standard deviation	0.05	0.05	0.06
Average recovery, %	90	85	86
Coeff. var., %	10	11	13
Manganese			
Average	5.14	5.24	5.04
Standard deviation	0.46	0.47	0.50
Average recovery, %	93	96	102
Coeff. var., %	9	9	10
Iron			
Average	36.96	37.17	37.02
Standard deviation	2.59	2.97	2.96
Average recovery, %	94	98	95
Coeff. var., %	7	8	8

N.B.: Standard deviation was estimated from twelve replicates for each metal.

in analysis, it was then necessary to evaluate the reliability and accuracy of the results obtained in this study using $HNO_3—H_2O_2$ sample digestion and flame atomic absorption spectrophotometry by comparing these with those obtained with $HNO_3—H_2O_2$ and inductively coupled argon plasma–atomic emission spectrometry. Table II shows the statistical data obtained. The student *t*-test at 95% confidence level was carried out on these values using the flame atomic absorption spectrophotometry as the "true" concentration. Though this showed that there is an apparent difference between these results, yet from inspection one would say that there is no significant difference between them. The relative deviation between these techniques is in the order of 10% and less for most metals studied.

The correlation coefficient for most of the metals studied showed a value close to unity which is an indication that there is good correlation or agreement between the flame atomic absorption spectrophotometry and the inductively coupled argon plasma–atomic emission spectrometry values. The good agreement between the

Table II Comparison of flame AAS with ICP–AES techniques in the determination of trace metals in fish samples

Metal	Conc. in μg/g mean of ICP–AES[a]	Correlation coefficient	% Relative deviation
Cu	2.59 ± 0.29 (2.81 ± 0.27)[b]	0.995	8
Zn	7.72 ± 0.77 (7.58 ± 0.92)	0.994	2
Pb	0.59 ± 0.08 (0.62 ± 0.07)	0.912	5
Ni	0.12 ± 0.01 (0.11 ± 0.01)	0.630	8
Mn	0.84 ± 0.08 (0.94 ± 0.08)	0.798	10
Fe	20.77 ± 1.63 (19.83 ± 1.79)	0.996	5

[a] Twelve replicates.

[b] Flame AAS values are in brackets.

flame atomic absorption spectrophotometry and the inductively coupled argon plasma–atomic emission spectrometry coupled with the good recoveries by the $HNO_3—H_2O_2$ procedure confirm the suitability of the $HNO_3—H_2O_2$ system and the flame atomic absorption spectrophotometry for trace metal determination in fish and shellfish samples. This was subsequently used in the determination of trace metals in fish and shellfish samples collected from the Niger delta area of Nigeria. The average concentration and range of metals in the fish and shellfish samples analysed are listed in Table III. The average trace metal concentrations in these samples are low when compared with the World Health Organisation (WHO) acceptable limit[14] for metals in foods, although the metal levels in some samples do approach or exceed WHO recommended limits.

Table III Average concentration and range of metals in fish and shellfish (first figure mean, second figure range) from the Niger Delta Area of Nigeria

Metal	Conc. in μg/g wet weight			
	Fish[a]		Shellfish[b]	
Cu	0.69,	0.02– 3.69	5.96,	3.30–13.43
Zn	4.80,	1.03–17.85	11.20,	5.35–16.60
Pb	0.48,	0.02– 1.98	1.40,	0.02– 6.40
Ni	0.28,	0.01– 1.09	0.48,	0.08– 1.79
Mn	1.11,	0.08– 6.90	3.17,	0.62–12.77
Fe	5.85,	0.49–16.52	28.26,	4.47–57.15

[a] = 61 samples.
[b] = 22 samples.

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