Full Length Research Paper

Modification and validation of a microwave-assisted digestion method for subsequent ICP-MS determination of selected heavy metals in sediment and fish samples in Agusan River, Philippines

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This study investigated, validated, and applied the optimum conditions for a modified microwave-assisted digestion method for subsequent ICP-MS determination of mercury, cadmium, and lead in two matrices relevant to water quality, that is, sediment and fish. Three different combinations of power, pressure, and time conditions for microwave-assisted digestion were tested, using two certified reference materials representing the two matrices, to determine the optimum set of conditions. Validation of the optimized method indicated better recovery of the studied metals compared to standard methods. The validated method was applied to sediment and fish samples collected from Agusan River and one of its tributaries, located in Eastern Mindanao, Philippines. The metal concentrations in sediment ranged from 2.85 to 341.06 mg/kg for Hg, 0.05 to 44.46 mg/kg for Cd and 2.20 to 1256.16 mg/kg for Pb. The results indicate that the concentrations of these metals in the sediments rapidly decrease with distance downstream from sites of contamination. In the selected fish species, the metals were detected but at levels that are considered safe for human consumption, with concentrations of 2.14 to 6.82 µg/kg for Hg, 0.035 to 0.068 µg/kg for Cd, and 0.019 to 0.529 µg/kg for Pb.

Key words: Mercury, cadmium, lead, CRM, pollution, dissolution, analysis.

INTRODUCTION

Although, standard methods of chemical analyses are available from many recognized sources, there are occasions when they cannot be used or when their use is inconvenient, e.g., when no standard method exists for a particular constituent or characteristic, such as extremely low heavy metal concentrations. In most analytical procedures the sample is physically destroyed by dis-solution, calcinations or by other digestion methods (Quevauviller, 1993). This stage of analysis can possibly cause losses of the analytes and correspondingly affect the accuracy and precision of the final results obtained. The accuracy can be determined by extracting a relevant certified reference material (CRM) and calculating the percentage recoveries relative to the certified values. The use of CRMs is also a good way to identify losses due to the method used. Several sample dissolution methods are commonly used for heavy metal analyses, including open vessel and microwave-assisted digestions. In the past few years, the latter has proven to be a useful tool in sample preparation for both organic and inorganic analytes.

This technique is advantageous compared to other

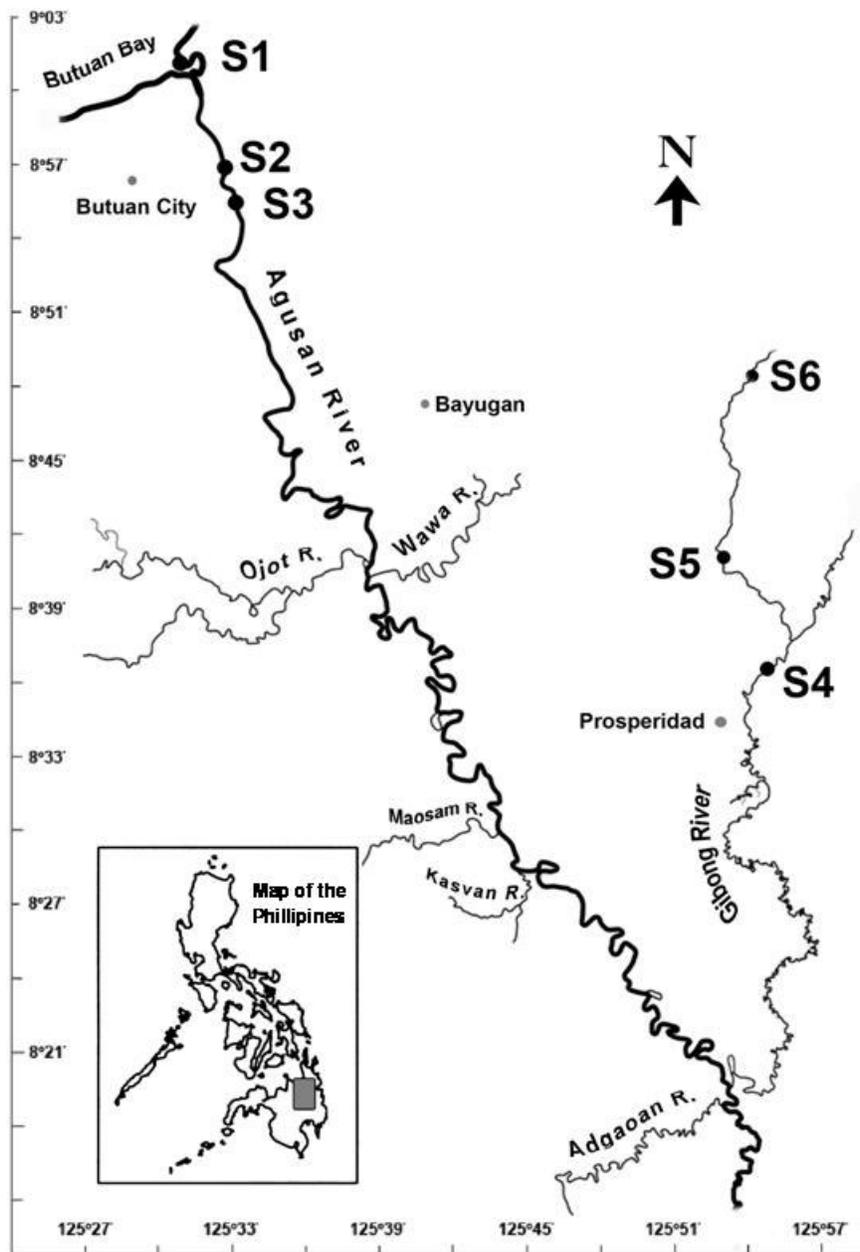*Corresponding author. E-mail: bebottcroa@yahoo.co.uk.

Figure 1. Location of study sites within the Agusan (S1 to S3) and Gibong (S4 to S6) Rivers, in northeastern Mindanao, Philippines.

methods as it provides rapid sample preparation and reduces the risk of contamination. The group of Shalini Ashoka (2009) presented a comparison of six acid digestion methods involving nitric acid in conjunction with other reagents to digest three certified marine biological samples (DOLT-3, DORM-3, IAEA-407) and a fish bone homogenate (prepared from *Merluccius australis*) for subsequent ICP-MS determination of 40   elements. Results of their study showed that microwave digestion with nitric acid and hydrogen peroxide (Method III) gave the   most   acceptable   results   as   determined   by

comparison with the certified values. Deshpande et al. (2008) used nitric acid and perchloric acid in digesting fish samples (including mackerel, pomfret, king fish and Indian salmon collected from Mumbai Docks) on a hot plate and further reconstituted the digest using HCl for subsequent analysis using a voltametric analyzer.

The U.S. EPA Method 3051 is a microwave-assisted digestion method and is known to be a regulatory alternative to the earlier microwave-assisted Method 3050 (Lorentzen and Kingston, 1996; U.S. EPA, 1995) that provides rapid, safe, efficient digestion that is invulnerable

to losses of volatile metallic analytes (Hewitt and Reynolds, 1990). A modification of this method, U.S. EPA Method 3051A, is also a microwave-assisted digestion method for sediments, sludges, soils, and oils for trace metal analysis. However, this method has some conditions that cannot be applied using some types of commercial laboratory microwave digestors. For example, this method specifies 175°C temperature that corresponds to approximately 14 - 17 atm or 205 - 250 psi (U.S. EPA, 1997) depending on the type of sample used. This pressure range exceeds the 200 psi maximum allowable pressure for CEM Microwave Oven (CEM Corporation, Matthews, NC, USA). U.S. EPA Method 3052 is another microwave-assisted digestion designed for digesting siliceous and organically based matrices. In this method, hydrofluoric acid (HF) is added at varying concentrations depending on the silicon dioxide (silica, SiO_2) concentration in the sample. Obviously, it would be attractive to use a method that does not require the use of dangerous HF. Open vessel digestion methods can also be applied to analysis of heavy metals such as cadmium (Cd) and lead (Pb) but are not applicable to the volatile analyte mercury (Hg). Thus, closed digestion systems are preferred to minimize possible contamination of the digest and avoid losses of volatile elements.

Several analytical instruments have been used in the final determination of the analyte concentrations in different environmental matrices. For instance, flame and electrothermal atomic absorption spectrometry (ET-AAS) are not used for the analysis of Hg in sediments because of very poor detection limits but can be used when coupled with pre-concentration steps (Ciceri, 2008). Later, it was reported that ET-AAS has improved its limits of detection for As, Se, Pb, Te, Cd, Sn, Sb, Bi, and Au, as well as Hg by employing chemical vapor generation and in-atomizer trapping techniques (Lampugnani et al., 2003; Moscoso-Pérez et al., 2003). Then, ICP-OES with fast multi-element technique was also used, as it has dynamic linear range, although with low to moderate limits (Sturgeon, 2000). The problem of low to moderate detection limits was overcome by using ICP-MS. The ICP-MS offers high sensitivity and pre-concentration steps are not even mandatory (Ciceri et al., 2008).

The aims of this study were: (1) to modify a microwave-assisted digestion technique for subsequent ICP-MS determination of Hg, Cd, and Pb from various environmental matrices; (2) to validate the method using CRMs for sediment and fish samples; and (3) to apply the validated method in the analysis of sediment and fish samples from Agusan River and Gibong River, a tributary of the Agusan River, in Agusan del Sur, eastern Mindanao, Philippines (Figure 1).

The geographical locations of both rivers in Figure 1 suggest that they are carriers and passageways of effluents draining from surrounding areas, which are involved in either or both agricultural and industrial activities, predominantly artisanal gold mining. Gibong River merges with Agusan River, which finally empties to

Butuan Bay. Appleton et al. (2006) reported that Hg, Cd, and Pb concentrations of 27 to 124, <1-5, and 14 to 186 mg/kg, respectively, were observed in the suspended solids, and 17 to 33, < 1, and 17 to 19 mg/kg in bottom sediment samples collected from the upstream region of Agusan River below Naboc River whose headwater was the focus of the mining activity in Mt. Diwalwal (Appleton et al., 2006). However, the impact of similar activities on other tributaries of Agusan River has not been well documented. Thus, the validated microwave-assisted digestion method of this study was applied to sediments and fish samples collected from several locations along both Agusan River and Gibong River to determine the effects of human activities in these areas.

MATERIALS AND METHODS

Certified reference materials

The CRM for sediments in this study, coded as NCSDC 73372, contains certified values of 60 elements including Hg, Cd, and Pb, obtained from the mean of 14 independent laboratories, provided by the Institute of Geophysical and Geochemical Exploration (China) to China National Analysis Center for Iron and Steel, and was distributed by Graham B. Jackson PTY LTD, Dandenong 3175 Victoria. This CRM is a lake deposit in powdered form with size less than 0.074 mm.

The fish CRM, coded as LUTS-1, is a non-defatted lobster hepatopancreas reference material for 16 trace metals including Cd and Pb (National Research Council, Canada). The certification process of this CRM was verified by 5 other external expert laboratories.

Standards and reagents

The standards for ICP-MS were prepared from stock solutions of Hg, Cd, and Pb at 1000 mg/L concentrations obtained from Sigma-Aldrich, Australia, and labelled as Fluka TraceCert Ultra. Spiked solutions were prepared from the stock as necessary. Ultrapure nitric acid (HNO_3) and hydrochloric acid (HCl) reagents were obtained from J.T. Baker Inc. All other reagents and solvents used in this study were analytical grade obtained from Sigma-Aldrich, Australia. All water used in washing laboratory glassware, other apparatus, and in the preparation of sample and standard solutions were deionized (resistance < 18 mΩ, Academic Milli-Q Ultra Pure Water System, Australia).

Instruments and apparatus

All microwave-assisted digestions were performed using CEM MDS 2000 630 WATT system (CEM Corporation, Matthews, NC, USA). This system was designed to hold 12 polytetrafluoroethylene (PTFE) digestion vessels. The digestion vessels had rupture membranes for safe operation under 200 psi. The elemental analyses were conducted using an Agilent 7500ce inductively coupled plasma mass spectrometer (ICP/MS) (AgilentTechnologies, USA). Optimization of the instrument was done as outlined in the Agilent 7500ce user's manual. The operating conditions of the instrument are shown in Table 1. This type of ICP-MS is fitted with an octopole reaction-collision cell, operated in collision mode that will minimize the isobaric interferences from polyatomic ions such as oxides (MO^+) that may occur from either the matrix or the plasma

Table 1. Operating conditons of the Agilent 7500ce ICP-MS (AgilentTechnologies, USA).

Operating conditions	Value
Plasma gas flow rate (L/min)	13
Auxiliary gas flow rate (L/min)	1.5
Carrier gas flow rate (L/min)	0.85
Makeup gas flow rate (mL/min)	0.28
Collision gas/flow rate (mL/min)	He 4.2
RF power (W)	1550
Nebuliser	Micro mist
Torch injector internal diameter (mm)	2.5
Sample depth (mm)	8.0
Interface cone	Pt sampler cone, Ni skimmer
CeO^+/Ce^+ (%)	0.5

Table 2. Microwave-assisted digestion conditions used in the study.

Stage	Power (%)	Pressure (psi)	Total time (min)	Time at that pressure(min)
		Set I[a]		
1	75	75	35	30
2	100	75	20	15
3	60	20	35	30
		Set II[b]		
1	40	20	10	5
2	40	40	10	5
3	40	85	10	5
4	40	135	10	5
5	40	175	10	5
		Set III[c]		
1	70	20	10	5
2	100	40	10	5
3	100	85	10	5
4	100	135	10	5
5	90	175	10	5

[a]Set I has 3 stages with variable percent power (75%, 100%, 60%) and pressure (20 to 75 psi) for a total digestion time of 90 minutes. [b]Set II is designed   for digesting 2 sample vessels and has 5 stages with constant percent power (40%) but different pressures (20 to 175 psi) for a total digestion time of 50 min. This was based from Cem-Microwave sample preparation note for digestion of fish tissue in a closed vessel using pressure controlled microwave heating for the determination of metals by spectroscopic methods. [c]Set III has also 5 stages with variable percent power (70%, 100%, 100%, 100%, 90%) and variable pressure (20 to 175 psi).

through kinetic energy bias. Additionally, this instrument is extremely sensitive, with a very large dynamic range that can measure analytes from the very low ng/L (ppt) level to the upper mg/L range (1000 mg/L) in one run or a total range of 8 to 9 orders of magnitude.

Modification and optimization of the microwave-assisted digestion method

In this study, three different sets or combinations of power, pressure, and time conditions for microwave-assisted digestion (Table 2) were tested using the two CRMs representing sediment and fish to determine the optimum set of conditions. Set I was based from the work of Lim et al. (2007) which determined the effects of fuel characteristics and engine operating conditions on elemental composition of emissions from twelve heavy duty diesel buses in Brisbane, Australia; Set II was based on the method outlined for CEM Microwave Sample Preparation System (Microwave sample preparation note for digestion of fish tissue in a closed vessel using pressure controlled microwave heating for the determination of metals by spectroscopic methods) (CEM Corporation, Matthew, NC, USA). This method was described for use with only 2 vessels (5% power was added for every increase of sample vessel), so Set III is a modification of Set II (by this study) using 12 microwave vessels simultaneously.

These three sets of microwave-assisted digestion conditions involved the digestion of 0.5 g of the CRM with 10 mL HNO$_3$ in a CEM microwave oven. After digestion, the vessels were allowed to cool until the pressure of the vessel was reduced to below 50 psi. The caps of each vessel were then carefully removed and the contents were filtered using 0.45 µm microfibre filter (Banksia Scientific, Australia), diluted to 20 mL in a volumetric flask using deionized water, and stored in polyethylene vial prior to the final determination of the trace metals' concentration.

Validation of the optimized microwave-assisted digestion method

From the three sets of conditions tested, the one that gave the highest recovery (the optimum set) was validated using three validation techniques. First, the three CRMs were digested and the percentage recoveries were compared to the certified values of the CRM. In the case of LUTS-1, which does not include certified value for mercury, 400 µL of a 1000 mg/L (or 400 µg) Hg standard was spiked to the CRM prior to digestion. The second and third techniques for validating the microwave methods were by comparing the recoveries to recoveries obtained when the same CRMs were analyzed using two standard methods: AOAC Method 990.08 for Cd and Pb and EPA Method 200.7 for Hg, Cd, and Pb, respectively. These two standard methods are both open vessel digestion procedures with the temperature maintained at 95°C. In the validation relative to EPA Method 200.7, the three CRMs were spiked with 400 µL of 1000 mg/L (or 400 µg) each of Hg, Cd, and Pb standards. Spiking was done to elevate the analyte concentration in the CRMs and particularly to supply Hg to LUTS-1.

Detection limits

The minimum detection (MDL) and instrument detection limits (IDL) were calculated for the three elements (Hg, Cd, and Pb) as 3 times the standard deviation of the concentrations of the seven blanks, and sum of the MDL and mean metal concentration of the blank, respectively (Agilent Technology, 2005).

Application of the validated microwave-assisted digestion method

Study sites

Sediment samples were collected from three sites each in Agusan River and in Gibong River (Figure 1) on April 16, 2009. Agusan River is located in the eastern part of Mindanao Island in the Philippines. It has several creeks and river tributaries and has become the recipient of waste deposits carried by Naboc River which receives wastes from mine tailings of Mt. Diwalwal, the biggest and most controversial gold mining area in the Philippines and a significant global gold producer (Appleton et al., 2006). Gibong River is one of the major river tributaries of Agusan River and carries both agricultural and industrial effluents and wastes from Agusan del Sur. The basis for selecting the sites was the absence of data comparing the selected trace metals' (Hg, Cd, Pb) concentrations in Agusan River and its tributary, the Gibong River.

Types of sample and sampling protocol

Approximately 2 kg sediment samples were collected at a depth of approximately 2 ft. from the designated sampling sites using a modified corer made of PVC pipe (2 ft. × 6 in i.d.) and wet-sieved *in situ* using a screen with an opening of $^1/_8$ inch. This was done to remove the twigs, leaves, rocks and other materials that were incorporated and collected together with the sediment samples in the corer. The collected sediments were allowed to stand for 1 h in a basin and were then sieved to separate from the water. The sieved sediments were kept in polyethylene bags, stored in an ice chest, and transported to the MSU-IIT Chemistry Laboratory. In the laboratory, the sediments were freeze-dried, ground, and sieved to obtain a particle size of < 75 µm. Fish samples were bought from the fishermen fishing near Study Site 1 (SS1) during the time when sediment sampling was done. The samples included 5 pieces of *Arius maculatus* (sea catfish), 10 pieces *Ambassis commersonii* (glassfish), 10 pieces *Johnieops vogleri* (croaker fish), and 10 pieces *Sillago sihama* (common whitings). These fish species are all eaten in the local region and form an important part of the diet. In the laboratory, the meat was separated from the fishbone, then homogenized, freeze dried, transferred to polyethylene bags, and kept in desiccators until the analyses.

Analyses of mercury, cadmium, and lead

The operating conditions of the ICP-MS instrument were optimized before the analysis was performed. In particular, the nebulizer gas flow rate, the ion lens voltage(s) and the ICP RF power were adjusted to yield the highest signal intensities possible while maintaining low levels of oxides and doubly-charged ion production (both should be less than ~ 3%). After the instrument had been optimized, appropriate calibration standards were then measured. As part of the quality assurance protocol, at least six-point calibrations of different ranges (0.10 to 15.0 mg/L and 0.05 to 2000 µg/L) were carried out for Hg, Cd, and Pb to generate a calibration curve with correlation coefficient of 0.999 or better. The sample solution concentrations were then determined from the corresponding calibration curve. Calibration standards were also analyzed at regular intervals during analytical runs for the ICP-MS to ensure that the instrument continued to meet acceptable sensitivity and linearity criteria.

Data treatment and statistical analysis

Basic statistical parameters such as mean value, standard deviation, and relative standard deviation were calculated using EXCEL 2007 (Microsoft Inc., Redmond, USA) while the statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) (Version 12.0) software (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Performance evaluation of the microwave-assisted digestion method under different conditions

Figure 2 shows the percent recoveries of the three target analytes obtained during the performance evaluation using the three sets of microwave-assisted digestion conditions. It is noticeable in Figure 2 that higher percent recoveries of all target analytes from sediment CRMs were obtained using Set I conditions as compared to those obtained using Set II. On the other hand, the percent recovery of Hg, Cd, and Pb from fish CRM obtained using Set II was higher compared to those obtained using Set I. Note that, neither set of conditions
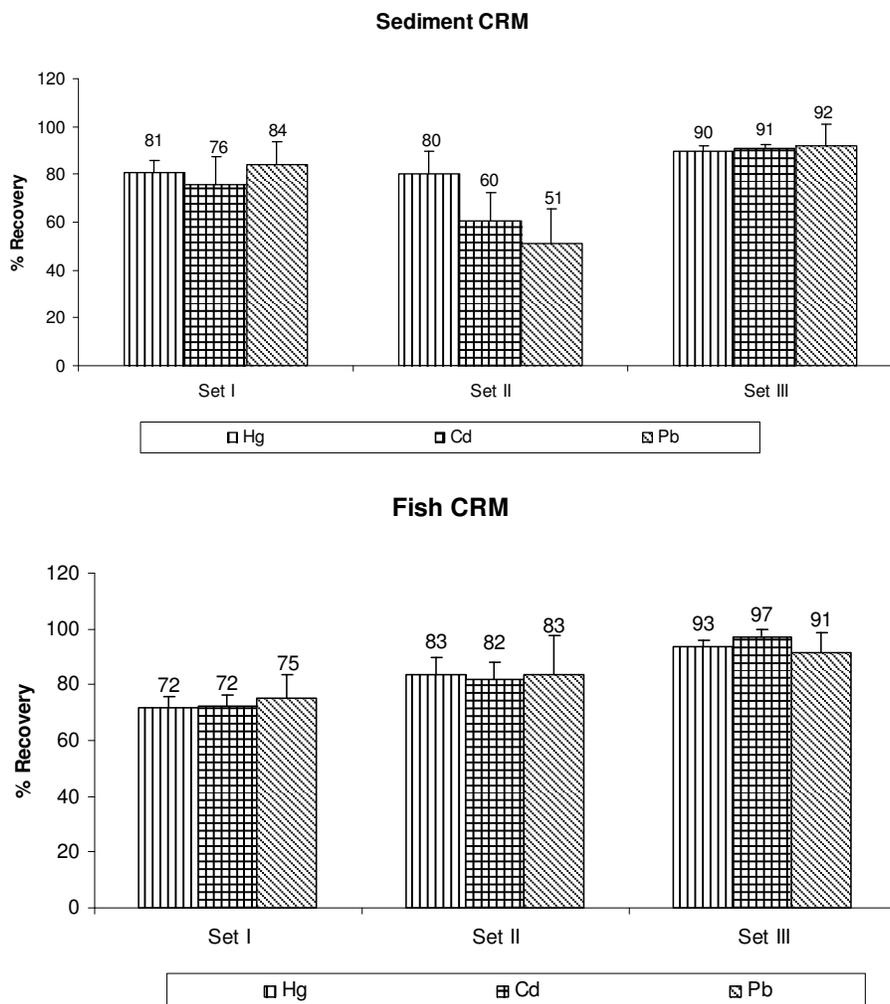
Figure 2. Percent recoveries of Hg, Cd, and Pb from sediment and fish CRMs using Sets I, II, and III microwave-assisted digestion conditions.

gave consistent and acceptable recoveries of all of the target analytes from the two different matrices. However, Set III, which was a modification (by this study) of Set II, gave improved and acceptable percent recoveries (90 to 93% Hg, 91 to 97% Cd, and 91 to 92% Pb) of all the target analytes from the two matrices.

Validation against certified reference materials: First validation technique

Table 3 shows the validation results of the modified and optimized microwave-assisted digestion method (Set III) using two different CRMs (sediment CRM, fish CRM). As shown in Table 3, the recoveries (in mg/kg) obtained using the method is in good agreement with the certified values of Hg, Cd, and Pb in the certified reference materials. For example, Cd in LUTS-1 CRM was recovered at a concentration of 2.06 ± 0.15 mg/kg while

its certified value is 2.12 ± 0.15 mg/kg. This translates to 97% accuracy at a precision of 2.6% RSD. Thus, the method also showed very good accuracy and precision as indicated by the values of the percent recovery (Column 4) and percent RSD of less than 10% (Column 5).

Validation against EPA Method 200.7 and AOAC Method 990.08: Second and third validation techniques, respectively

The accuracy of the method was further validated by comparing the percent recoveries from analysis of CRMs obtained using the modified and optimized microwave-assisted digestion method to those obtained using two standard methods - EPA Method 200.7 and AOAC Method 990.08 (Figure 3).

The results shown in Figure 3 indicate that the modified

Table 3. Validation results showing the percent recoveries[a+] (accuracy) and percent RSD (precision) of Hg, Cd, and Pb from sediment and fish CRMs as obtained using the modified and optimized microwave-assisted digestion methods.

Analyte	Certified value (mg/kg)	Microwave digestion validation results (Using the First Validation Technique)[b]		
		A (mg/kg±SD)	B (%)	C (%)
Sediment CRM: NCSDC 73372 (Lake deposits)				
Hg	0.030	0.027±0.001	90	1.9
Cd	0.10	0.091±0.002	91	1.8
Pb	25	23.024±2.212	92	9.6
Fish CRM: LUTS-1 (non-defatted lobster hepatopancreas)[c]				
Hg[c]	1000	934.600±26.888	93	2.9
Cd	2.12±0.15	2.057±0.054	97	2.6
Pb	0.010±0.002	0.009±0.001	91	8.2

[a]Relative to the certified values. [b]A - Recovered Concentration Values; B - Recovery (Accuracy); C - Relative Standard Deviation (Precision). [c]LUTS-1 does not have certified values for Hg. Thus, 400 µL of 1000 mg/l Hg (or 400 µg) was spiked to the CRM instead.

and optimized microwave-assisted digestion method achieved significantly better recovery values (91 to 93% Hg, 90 to 96% Cd, and 92% Pb) of all target analytes from the two different CRMs (sediment CRM, fish CRM) compared to the other methods (EPA Method 200.7: 89% Hg, 88 to 90% Cd, 86 to 90% Pb; AOAC Method 990.08: 85 to 90% Cd, 87 to 95% Pb;) ($p < 0.05$). For example, the Pb in CRM-NCSDC 73372 (lake deposits) was only 86 and 87% recovered using EPA Method 200.7 and AOAC Method 990.08, respectively, compared to 92% using the method being validated. Furthermore, the new method also showed very good precision and repeatability with % RSD values ranging from 1.8 to 9.6%.

In general, based from the results obtained such as percent recovery and precision (Table 3), the modified microwave-assisted digestion method (Set III conditions) has been validated to be more accurate and precise compared to the other two microwave methods (Set I and Set II) and the two open digestion methods (EPA Method 200.7 and AOAC Method 990.08) considered in the study. Thus, it can be used in the analysis of Hg, Cd, and Pb in different matrix types such as sediments and fish tissues using a single set of microwave digestion conditions. It should also be noted that the microwave method is quicker, cleaner, and easier to perform if a microwave is available, compared with the open digestion methods. Furthermore, this modified and vali-dated microwave-assisted digestion method combined with ICP-MS is able to extract and detect with very good precision and accuracy extremely low levels of heavy metal concentrations in sediment and fish matrices.

Detection limits

The minimum detection limits or method detection limits (MDL) for Hg, Cd, and Pb using the modified and validated

microwave assisted digestion were 1.409 µg/L for Hg, 0.001 µg/L for Cd, and 0.002 µg/L for Pb, while the instrument detection limits (IDL) were 1.750 µg/L for Hg, 0.002 µg/L for Cd, and 0.003 µg/L for Pb.

Application of the validated microwave-assisted digestion method

The newly validated microwave-assisted digestion method (Set III conditions) was subsequently applied in the analyses of the selected analytes (Hg, Cd, and Pb) in the sediment and fish samples collected from Agusan and Gibong Rivers. Tables 4 and 5 show the mean concentrations of Hg, Cd, and Pb observed in sediments and fish samples collected from these study sites, respectively.

Concentrations of mercury in sediments

An extremely high mean Hg concentration (341.06 mg/kg) was observed in Study Site 6 (SS6) ($p < 0.05$) which is located approximately 5 m from the drainage of the previously operated plant for gold extraction by amalgamation followed by SS5 (69.78 mg/kg Hg) which is approximately 40 km from SS6 (Figure 1 and Table 4). The Hg concentration dropped significantly from SS6 downstream towards the mouth of the Agusan River; the three sites near the mouth of the river have similar, much lower values. A similar trend has been reported by Appleton et al. (1999), indicating that in the Agusan River basin, elevated sedimentary Hg concentrations occur close to point sources.

Note that this is the highest Hg concentration observed in this study and is much higher than the previously reported 27 to 124 mg/kg Hg in bottom sediment samples collected in Agusan River (Appleton et al., 2006).
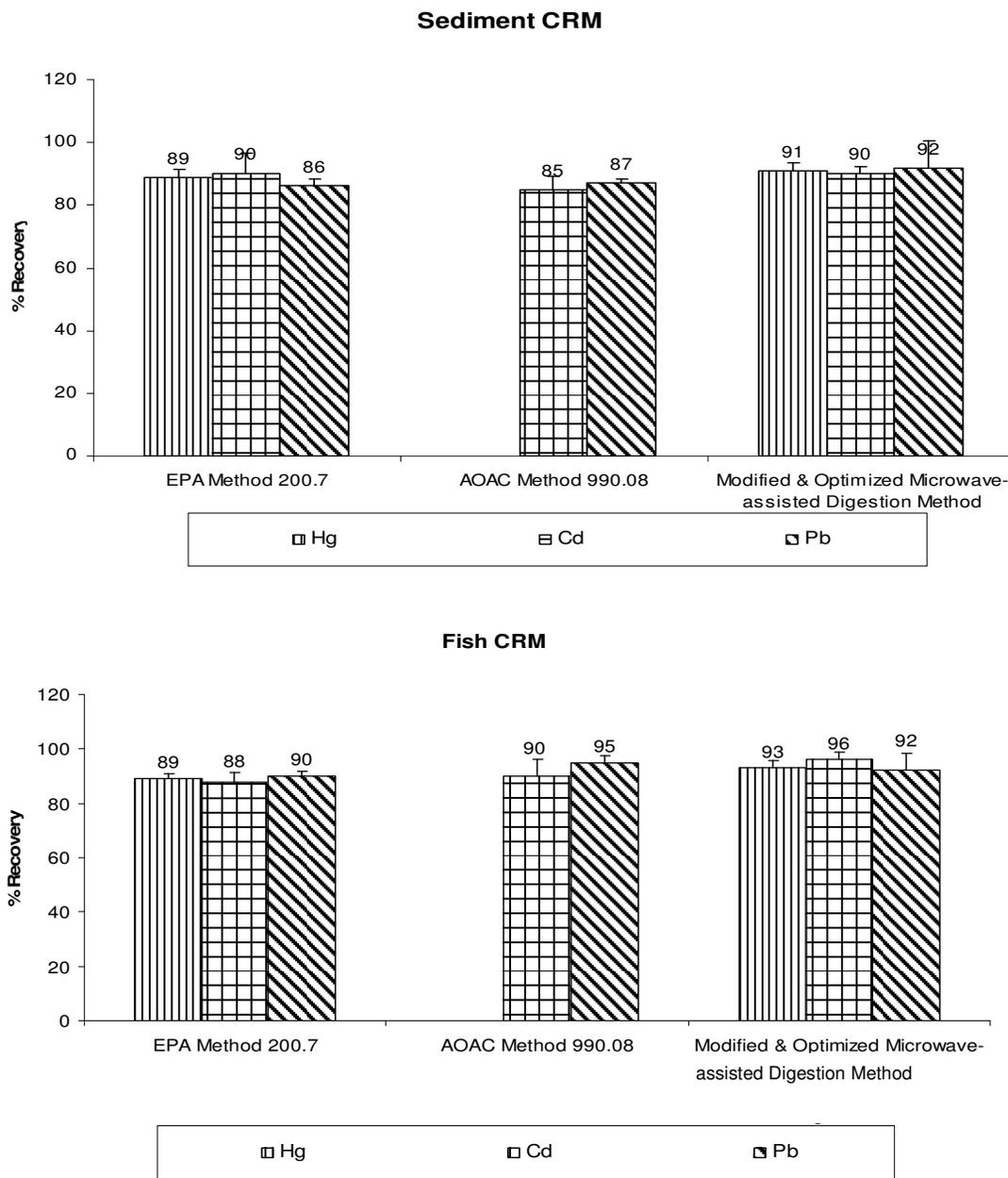
Sediment CRM

Fish CRM

Figure 3. Comparison of percent recoveries (accuracy) of Hg, Cd, and Pb from certified reference materials (sediment and fish) using EPA Method 200.7, AOAC Method 990.08, and the modified and optimized microwave-assisted digestion method. Note that in AOAC Method 990.08, Hg is not included in the analysis.

However, that part of Agusan River, as mentioned in the introduction, is further upstream than the stretch of the river included in this study. Of significance, the Hg concentrations found in sediments at all six sites in the Agusan and Gibong Rivers exceed the permissible threshold effect level of 1 mg/kg Hg set by Environment Canada 1999 (Haines et al., 1994; CCME, 1999), Federal Water Quality Administration Criterion for dredge material, the maximum acceptable concentration in the United States (Pavlou et al., 1987), and Sediment Quality

Guidelines in Australia and New Zealand (ANZECC, 1998).

Concentrations of cadmium and lead in sediments

In this study, the Cd and Pb concentrations found in sediments range from 0.05 to 44.46 mg/kg and 2.20 to 1256.16 mg/kg, respectively. Again, the highest concentrations of these metals were found at S6; Cd and

Table 4. Mean (± SD) and percent RSD of Hg, Cd, and Pb concentrations (mg/kg dry weight) in sediments from selected sites in Agusan River and Gibong River as obtained using the validated method*.

Study site (Location)	Hg (mg/kg±SD)	%RSD	Cd (mg/kg±SD)	%RSD	Pb (g/kg±SD)	%RSD
SS1 (N 9.01548º) (E125.51810º)	$13.92^b \pm 0.39$	2.81	$0.22^a \pm 0.003$	1.33	$7.25^a \pm 0.42$	5.80
SS2 (N 8.94703º) (E125.54669º)	$5.69^a \pm 0.30$	5.32	$0.05^a \pm 0.001$	2.30	$2.20^a \pm 0.09$	4.27
SS3 (N 8.92985º) (E125.55300º)	$2.85^a \pm 0.12$	4.39	$0.37^a \pm 0.018$	4.85	$11.51^a \pm 0.49$	4.28
SS4 (N 08.61035º) (E125.91387º)	$8.86^{ab} \pm 0.29$	3.30	$0.07^a \pm 0.001$	0.75	$3.53^a \pm 0.10$	2.68
SS5 (N 08.68178º) (E125.66371º)	$69.78^c \pm 1.84$	2.63	$0.66^a \pm 0.020$	2.96	$17.23^a \pm 1.07$	6.22
SS6 (N 08.81667º) (E125.90000º)	$341.06^d \pm 6.76$	1.96	$44.46^b \pm 1.579$	3.55	$1256.16^b \pm 50.07$	3.99

*Note: Lower case superscript indicates *anova* result at α=0.05. (The same superscripts written in the same column indicate that the corresponding values have no significant differences)

Table 5. Mean (± SD) and percent RSD of Hg, Cd, and Pb concentrations (µg/kg dry weight) in fish samples from Agusan River (near SS1) as obtained using the validated method.[*]

Sample	Hg, µg/kg±SD	% RSD	Cd, µg/kg±SD	% RSD	Pb, µg/kg±SD	% RSD
Arius maculatus	$6.82^a \pm 0.26$	3.79	$0.440^a \pm 0.030$	6.86	$0.529^a \pm 0.044$	8.38
Ambassis commersonii	$3.51^b \pm 0.18$	5.02	$0.035^b \pm 0.003$	8.39	0.151 ± 0.008	5.44
Sillago sihama	$2.22^c \pm 0.06$	2.56	$0.068^b \pm 0.002$	2.47	$0.029^c \pm 0.003$	8.83
Johnius vogleri	$2.14^c \pm 0.13$	6.24	$0.037^b \pm 0.001$	3.69	$0.019^c \pm 0.001$	1.66

[*]Note: Lower case superscript indicates *anova* result at α = 0.05. (The same superscripts written in the same column indicate that the corresponding values have no significant differences).

Pb concentrations were significantly lower in the other study sites (Table 4). There is no significant trend observed for sediment data from SS5 going towards SS1, which is situated at the mouth of Agusan River. Thus, the proximity of SS6 to the point source is presumably the major factor responsible for the highest concentrations of all metals observed in this site. Birch and Taylor (1999) reported a Pb concentration as high as 1000 µg/g (1000 mg/kg) and a moderate concentration of >2 µg/g (2 mg/kg) for Cd in superficial sediments collected from Port Jackson Estuary, Australia. The distribution of these metals exhibits a strong declining trend in concentration from the upper reaches of the estuary towards the estuary mouth (Birch and Taylor, 1999).

IMPLICATIONS FOR THE ENVIRONMENT

The sediment samples collected from both Agusan and Gibong Rivers contain detectable concentrations of the selected hazardous heavy metals Hg, Cd, and Pb. The distribution of these metals showed a strong decreasing pattern in concentration from the upper reaches of the river towards the river mouth. The heavy metal pollution in both rivers drops off with distance from a polluting source, confirming the old adage that "the solution to pollution is dilution". It also shows an observable close relationship between the land use in the catchment area and the concentration of the heavy metal. The highest concentration of all metals was observed in SS6 which is situated in the headwater of Gibong River, and which is the location of one of the mining establishments in the area. Thus, waste discharged from mining industries is a major point source of these metal pollutants suggesting that their presence is anthropogenic in nature.

Concentrations of mercury, cadmium, and lead in selected fish species

The Hg concentrations in the four fish species considered in this study (Table 5) were found to be 2.14 to 6.82 µg/kg. This range of Hg concentrations is significantly lower than the 500 µg/kg maximum recommended limit for Hg (UNEP, 2002) commonly allowed in fish for human consumption in most countries. It is also lower than the findings of Maramba et al. (2006) for Hg concentrations in marine biota (1.65 to 1152.01 µg/kg) from three communities near abandoned gold mines in Zamboanga del Norte, Philippines. As shown in Table 5, Cd values were found to be in the range of 0.035 to 0.44 µg/kg. EU and Turkish Limits for Cd are the same value (0.1 mg/kg) while the World Health Organization specifies a 0.5 mg/kg maximum permissible Cd content in fish (EU, 2001; FAO/WHO, 1987). Note that the Cd content in *A. maculatus* is significantly higher than in the other three fish species, but still much lower than these allowed limits.

The Pb concentration detected in the four fish species ranged from 0.019 to 0.53 µg/kg. This range of Pb concentrations in fish is significantly lower than the maximum permissible and tolerable values of 0.1 mg/kg Pb and 0.5 mg/kg Pb, set by EU 2001 and FAO/WHO 1987, respectively, and does not demonstrate risk if utilized for human consumption. Note that the highest concentration was exhibited in the same fish species (*A. maculatus)* whose Cd and Hg concentrations were also the highest. *A. maculatus* is carnivorous and a bottom-dweller. The adults of *A. maculatus* feed on insect larvae, shrimps, worms, fish and whatever organic debris that occur on the bottom (Conlu, 1986). Two of the other species tested, *J. vogleri* and *S. sihama* are also bottom dwellers but do not feed on detritus. Presumably, ingestion of detritus is increasing the levels of heavy metal in the tissues of this fish.

The Hg, Cd, and Pb values in the fish species in this study (2.14 to 6.82 µg/kg Hg, 0.035 to 0.44 µg/kg Cd, 0.019 to 0.53 µg/kg Pb) are much lower than the reported Hg, Cd, and Pb levels in *Tilapia spp.* (0.125 to 0.277 µg/g Hg, 0.13 µg/g Cd, and 0.16 µg/g Pb) and *Taiwan clams* (0.315 to 0.869 µg/g Hg, 0.12 to 0.16 µg/g Cd, and 0.22 to 0.24 µg/g Pb) collected in Naboc and Agusan Rivers (Appleton et al., 2006) upstream of where this study was completed. Thus, based on the Hg, Cd, and Pb levels de-termined in the four fish species considered in this study, it can be concluded that all fish species in the area are safe for human consumption. These results showed that fortunately, in spite of industrial activities along the two rivers, fish that is being consumed has tolerable heavy metal concentrations that should not affect the health of the human consumers.

Metal intake related to fish consumption

Fish is one of the most indicative factors in freshwater system for estimating heavy metal pollution and risk potential to human health (Papagiannis et al., 2004). From the results of this study, a consumption of 1 kg of fish per week from Agusan River is equivalent to intakes of 2.14 to 6.82 µg Hg, 0.035 to 0.068 µg Cd, and 0.019 to 0.53 µg Pb per week (Table 5). These Hg, Cd, and Pb intakes per week are several folds lower than the Provisional Tolerable Weekly Intake (PTWI) of 300 µg Hg (FAO/WHO, 2003), 420 µg Cd (WHO, 2006), and 1470 µg Pb (WHO, 2000) recommended by the Joint FAO/WHO Expert Committee on Food Additives. Also, the reported weekly Hg intake of 277 µg Hg by a 60 kg person eating 250 g of fish four times in a week from Naboc River (Appleton, 2006) is enormously greater than the weekly Hg intake obtained here.

ACKNOWLEDGEMENTS

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