Analytical method validation for total mercury quantification in sediments using a Direct Mercury Analyzer

Adriana Esquivel[1], Kerling Rodríguez[1]

[1] *Institute of Studies on Toxic Substances of the Universidad Nacional (IRET-UNA), Heredia, Costa Rica*

ABSTRACT
A method for quantifying total mercury (Hg_T) in sediment samples has been validated using a Direct Mercury Analyzer (DMA). The validation was carried out according to the Eurachem Guides and the International Union of Pure and Applied Chemistry (IUPAC) validation guidelines. Linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability, intermediate precision, trueness, expanded uncertainty, robustness, and matrix effects were evaluated. Certified reference materials of channel sediment and collected sediment samples were used for the method validation. The linear range was 83–4855 µg/kg, with a LOD of 27 µg/kg and a LOQ of 83 µg/kg. Repeatability was 1.7 %–2.6 % at two concentrations tested, and intermediate precision was between 0.2 % and 0.4 %. The robustness of the method was assessed using the Youden test, which demonstrated that variations in experimental factors did not significantly influence the results. Furthermore, no significant matrix effects were observed. A proficiency test was also performed, with satisfactory outcomes. The results demonstrate that the method is reliable and suitable for the quantification of Hg_T in sediment samples.

1. INTRODUCTION

Mercury is a highly toxic environmental pollutant that is frequently found in crustal rocks of the Earth and coal deposits [1]. The harm from Hg pollution, coming from industrial activities and long-term atmospheric transport, has resulted in a sustained increase in Hg concentrations in the environment, affecting water sources, sediments, soil, and even human health [2].

Mercury can be released into the atmosphere from a variety of sources, usually due to chemical alterations in sediments and watercourses, which can further harm the ecosystem and public health. Both natural and anthropogenic processes transfer Hg into aquatic environments, where it transforms until it becomes available to living animals, leading to bioaccumulation and biomagnification phenomena, which, in turn, lead to affected animal health, the risk of species extinction, and human health through seafood consumption [3].

Excessive Hg in sediment can be converted by microorganisms into methylmercury, a highly toxic chemical that accumulates along the food chain. Mercury's toxicity to human health is directly correlated with its form [4].

Three types of Hg are found in nature: elemental mercury (Hg^0); inorganic mercury, which is mostly found as mercuric ($HgCl_2$, HgS) and mercurous (Hg_2Cl_2) salts; and organic forms, which include methyl [$(CH_3Hg)^+$] and ethylmercury ($C_2H_5Hg^+$). Elemental mercury is a highly volatile liquid element at room temperature and is frequently emitted into the environment as a vapour. Consequently, it can travel long distances and be deposited and sedimented in the oceans, either directly or through precipitation [3].

The World Health Organization (WHO) is concerned about the health and environmental risks of Hg. As a result of its danger, the United States Environmental Protection Agency (EPA) has developed air pollution and Hg guidelines, and the United Nations Environment Programme proposed the Minamata Convention on Mercury in 2013 [5].

The analysis of Hg in solid samples typically requires chemical pretreatments, such as acid extraction, pyrolysis [6], or other extraction techniques, which often generate hazardous waste and are time-consuming. Conventional methods, including gold amalgamation followed by cold vapour atomic fluorescence spectrometry (CVAFS) or spectroscopic analysis [7], also necessitate acid digestion and additional treatments to ensure accurate results [8]. In contrast, Direct Mercury Analyzers (DMAs), such as the Nippon MA-3000, enable direct determination of Hg without complex pretreatment, consequently simplifying the analytical workflow. These systems offer rapid analysis, high precision, and accuracy across diverse sample matrices, while their closed-system design minimizes contamination risks [9].

This study aimed to validate the performance of the Direct Mercury Analyzer Nippon MA-3000 for Hg sediment quantification, and evaluate precision, accuracy, and sensitivity. In addition, it sets out to compare the analytical response in solvent or sediment matrices and determine method applicability in environmental studies.

2. MATERIALS AND METHODS

2.1. Instrumentation

All analyses were conducted using a DMA (MA-3000, Nippon Instruments Corporation, Kyoto, Japan), which operates based on cold vapour atomic absorption spectrometry (CVAAS). The analyser is configured to measure absorbance at a wavelength of 253.7 nm and was operated using MA3Win software, version 2.4.4.

This technique involves the thermal decomposition of the sample to release Hg compounds, which are then reduced to elemental Hg. Any interfering species are removed during this process. The Hg vapour is captured via amalgamation on a gold trap, which selectively retains Hg, while eliminating any remaining interferences. Upon heating the trap, the Hg is desorbed and transported through dual absorption cells for quantification by CVAAS at 253.7 nm. This technique enables the determination of Hg_T in a wide range of matrices (solid, liquid) with minimal sample preparation. Quantification is based on the integrated peak intensity or peak area recorded during detection [10].

2.2. Glassware cleaning

All glassware was washed before use. Items were immersed in Extran MA05 detergent (Merck KGaA, Darmstadt, Germany) and sonicated in an ultrasonic bath (Model 5800, Emerson Branson, Danbury, CT, USA) for 30 minutes. After rinsing with water, the glassware was soaked in 1 % (v/v) nitric acid with sonication for 30 minutes, followed by another rinse with water. A final rinse was performed three times using ultrapure water (resistivity 18.2 MΩ·cm at 25 °C) obtained from a Merck Millipore A10 purification system (Merck KGaA, Darmstadt, Germany). The cleaned glassware was then heated in an oven (Heratherm, Thermo Scientific, Waltham, MA, USA) at 150 °C for 3 hours.

The ceramic sample boats were heated in a muffle furnace (Vulcan 3-550, Dentsply, Puerto Rico, USA) at 750 °C for 3 hours.

2.3. Chemicals and reagents

All working solutions were prepared by weighing the empty graduated flasks, aliquots, and final solutions. Concentrations were calculated by weight difference. A 100 mg/L Hg working solution was prepared by appropriate dilution of a certified Hg standard (1000 ± 4 mg/L in 12 % HNO₃, Sigma-Aldrich Production GmbH, Buchs, Switzerland) using 100 mg/L L-cysteine. Intermediate solutions of 1 mg/L and 10 mg/L were subsequently prepared and used to construct a calibration curve. Calibration standards were prepared in 100 mg/L L-cysteine (≥ 99.8 %, Nacalai Tesque, Kyoto, Japan). High-purity oxygen (99.5 %, Praxair, Alajuela, Costa Rica) was used as the carrier and purge gas. Certified reference material (CRM) BCR-320R (channel sediment; European Commission, Geel, Belgium) was used to assess recovery. A proficiency test with Axio PT-MT-472 was carried out.

2.4. Sampling

The sediments used were collected from a depth of 50 cm using an aluminium container and a small shovel, then transported in polytetrafluoroethylene (PTFE) containers. All samples were kept cold (4 °C) during transport to the laboratory, until analysis.

2.5. Sample preparation

The samples used were dried in an oven (Heratherm, Thermo Scientific, Waltham, MA, USA) at 50 °C until dry. Once dry, the samples were mortared and sieved through a 500 µm mesh (ASTM 35) to ensure homogeneity.

2.6. Sample analysis

For the analysis, 200 mg of dried sample or CRM was weighed using a five-digit analytical balance (Secura 125-1S, Sartorius, Germany) and transferred into ceramic sample boats for direct Hg determination using the MA-3000 analyzer.

3. RESULTS AND DISCUSSION

3.1. Method validation

The method validation was performed following the modified U.S. EPA Method 7473 [11], the Eurachem Guidelines [12], and the International Union of Pure and Applied Chemistry (IUPAC) guidelines [13]. The validation parameters included selectivity, matrix effect, linearity, working range, limits of detection (LOD) and quantification (LOQ), precision (repeatability and intermediate precision), trueness, robustness, and measurement uncertainty.

3.1.1. Selectivity

According to IUPAC (2001), "selectivity refers to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behaviour" [14].

The EPA Method 7473 provides high selectivity by incorporating a gold trap, which selectively captures and concentrates Hg vapour, effectively eliminating potential interferences, such as free chlorine or organic compounds. Detection at the Hg specific wavelength of 253.7 nm further minimizes the risk of spectral interference from other elements.

3.1.2. Matrix effect

Matrix effects were evaluated by spiking a representative sample at six concentration levels with a Hg standard solution. Four different samples of sediments were used. A matrix-matched calibration curve was constructed for each sample and compared with a standard calibration curve prepared in solvent. The results are summarized in Table 1. The slopes of both curves

Table 1. Analysis of variances between solvent and matrix curves.

Sample	Slope	Standard error	F_{cal}	t_{cal}
Solvent 1	2.53×10^{-3}	3.73×10^{-5}		
Solvent 2	2.55×10^{-3}	2.45×10^{-5}		
1	2.44×10^{-3}	3.20×10^{-5}	1.36	1.72
2	2.39×10^{-3}	1.12×10^{-4}	2.08	1.42
3	2.79×10^{-3}	1.09×10^{-4}	1.98	2.14
4	2.50×10^{-3}	5.99×10^{-5}	5.99	0.79

were statistically compared, using an F-test and a Student's t-test. F was calculated and compared with the tabulated F value ($F_{tab} = 6.39$) for the required confidence level ($\alpha = 0.05$), with 4 degrees of freedom in both the numerator and denominator, to assess differences in variance, and a Student's t-test was also performed [15]. The t value (t_{cal}) was calculated with $n - 2$ degrees of freedom for the required confidence level ($\alpha = 0.05$) with a two-tailed test [16] and compared with the tabulated t value ($t_{tab} = 2.31$) to evaluate the difference between slopes. If the F_{cal} and t_{cal} are below the tabulated value, there is no significant difference between variances and slopes. This approach enabled the assessment of matrix-induced signal enhancement or suppression, which could affect Hg quantification. Matrix 1 was compared with solvent 1, while matrices 2, 3, and 4 were compared with solvent 2.

3.1.3. Linearity and working range

A calibration curve with 6 levels and a blank was created using L-cysteine (100 mg/L) as solvent, measured approximately 12 times on different days.
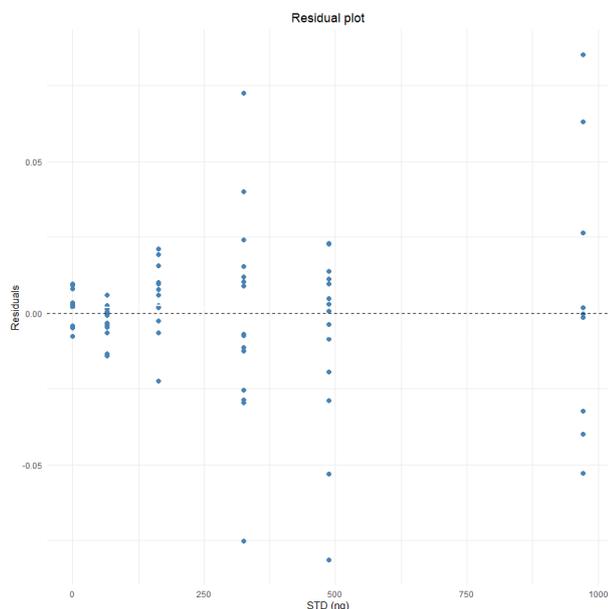

Figure 1. Plot of the residuals' random distribution.

Table 2. Difference of standard errors in the coefficients of the least squares linear regression.

	Coefficient		t-test coefficient	
	Estimate	Standard error	Estimate	Standard error
Slope	2.54×10^{-3}	9.95×10^{-7}	2.54×10^{-3}	1.39×10^{-5}
Intercept	9.02×10^{-3}	4.22×10^{-3}	9.02×10^{-3}	3.15×10^{-3}

Linearity was evaluated by a two-tailed Student's t-test to determine if there is a significant linear correlation for the required confidence level ($\alpha = 0.05$) [16]. The t value (t_{cal}) was calculated with $n - 2$ degrees of freedom and compared with the tabulated t value (t_{tab}) for the required confidence level ($\alpha = 0.05$) of a two-tailed test. In this case, n depends on the calibration levels. Equation (1) shows how t_{cal} was calculated:

$$t_{cal} = \frac{|r|\sqrt{n-2}}{\sqrt{1-r^2}}, \tag{1}$$

where $|r|$ is the absolute value of the correlation coefficient, $n - 2$ is the number of degrees of freedom, and r^2 is the value of the coefficient of determination.

The t_{cal} obtained was 41.64, and the tabulated t_{tab} was 2.78, which means there is a significant linear correlation with the calculated significance level.

Linearity was also evaluated based on a visual inspection plot of residuals. Random distribution of residuals around zero confirms linearity (Figure 1).

The second way for evaluation was using R Studio with the package 'lmtest', performing the studentized Breusch–Pagan test as a numerical method to detect heteroskedasticity, where a statistically significant p-value (0.0003791) indicates its presence [17]. To resolve heteroskedasticity, a regression analysis was performed to correct the issue of incorrect standard errors, so that interval estimates and hypothesis tests are valid. To achieve this, heteroskedasticity-consistent standard errors or simply robust standard errors are used. The concept of robust standard errors was suggested by Halbert White [18]. Using R Studio with the package 'sandwich', a t-test of coefficients was performed (Table 2). The working range was between 83 µg/kg and 4855 µg/kg with an R-squared equal to 0.999.

3.1.4. Limits of detection and quantification

The LOD and LOQ values were calculated according to the method described by IUPAC [19]. These limits were determined using equations (2) and (3):

$$LOD = 3.3 \cdot Sy/m \tag{2}$$

$$LOQ = 10 \cdot Sy/m, \tag{3}$$

where Sy is the standard error of the response and m is the slope of the calibration curve. The LOD obtained was 27 µg/kg and the LOQ was 83 µg/kg.

3.1.5. Precision

3.1.5.1. Repeatability

Repeatability was analysed over one day of analysis. The results were compared with the spiked concentrations (816 µg/kg and 4853 µg/kg). For repeatability, the % RSD experimental was calculated as the percentage SD of the mean value between the mean concentration value. The Horwitz´s coefficient of variation (% RSD$_H$) was used as an acceptability criterion. In both cases,

Table 3. Evaluation of the repeatability of seven replicates of a sediment sample at two different concentrations.

	Level 1 (816 µg/kg)	Level 2 (4853 µg/kg)
Mean (µg/kg)	842	4698
% RSD	2.1	2.4
% RSD$_H$	7	10

Table 4. The evaluation of intermediate precision by two analysts on different days.

Concentration spiked (µg/kg)	Mean concentration (µg/kg)	SD	RSD	Acceptability criteria Levene test (*p*-value)	Acceptability criteria ANOVA (*p*-value)
816	842	3.2	0.4	0.838	0.992
	842	2.5	0.3		
4853	4429	9.1	0.2	0.780	0.528
	4698	2.4	0.1		

the dispersion of the results was not significantly greater than the % RSD_H value. Table 3 presents the results obtained.

3.1.5.2. Intermediate precision

For the intermediate precision study, the results of the measurements performed by the two analysts on different days were used. For the statistical tests performed, Minitab19 was used.

Normality was determined using the Anderson–Darling test [20]. It was employed to detect deviations from normality in the data. It can be concluded that the samples met the normality condition because the *p*-value exceeds 0.05 at 816 µg/kg (0.793) and 4853 µg/kg (0.617).

A multiple comparisons test and a one-way ANOVA were performed to determine the homogeneity of variances and compare the means between analysts. Table 4 shows the results of the intermediate precision.

The assumption of the homogeneity of variances is verified through Levene's test [21]; if the *p*-value from Levene's test is below 0.05, it indicates the presence of differing variances. In both concentrations, the *p*-values were higher than 0.05, which means that there are no significant differences between variances (Table 4).

In the case of the one-way ANOVA test [22], there is no significant difference in means between analysts because the *p*-values are higher than 0.05 (Table 4).

3.1.6. Trueness

Trueness was evaluated by analysing seven replicates of the CRM BCR-320R (Channel Sediment, European Commission, Geel, Belgium). The results were compared with the certified value using the two-tailed Student's *t*-test (2.45) with 6 degrees of freedom at the required confidence level ($\alpha = 0.05$) [16] to determine if there is a significant difference in the concentration of the value obtained experimentally and the reference value of the CRM [23]. The t_cal was calculated using equation (4):

$$t_\text{cal} = \frac{[x_\text{a} - X]}{SD \cdot \sqrt{n}},$$ (4)

Table 5. The results of seven replicates for evaluating the trueness.

Parameter	Value obtained	Acceptability criteria
Mean (µg/kg)	847.14	850
SD	13	
Student *t*-test *value*	0.08	2.45
Bias (µg/kg)	2.86	45

where x_a is the concentration of the CRM, X is the mean concentration value obtained, SD is the standard deviation, and n is the number of replicates.

Additionally, the bias value was calculated. The results are shown in Table 5.

3.1.7. Robustness

Robustness was assessed by deliberately varying critical analytical parameters, such as sample weight, flow of oxygen, mortaring, sieving, homogenization, and the setup method, which could affect analytical performance (Table 6). Eight experiments were conducted, and the results were statistically analysed using the Youden and Steiner approach [24]. Table 6 and Table 7 summarize the findings, indicating that no variables had a significant effect on the results.

4. ESTIMATION OF MEASUREMENT UNCERTAINTY

The uncertainty measurement of the result was based on Eurachem Guide (2012) [11]. Measurement uncertainty can be estimated for Type A and Type B uncertainties. Type A is based on the statistical distribution of standard deviations of the mean replicates. Type B can also be characterized by standard deviations, evaluated from probability density functions based on experience or other information. Table 8 represents Type A and Type B uncertainties. The combined relative standard uncertainty (u_c) was calculated as the sum of the squared of relative standard uncertainties u from equation (5)

$$u_\text{c} = \sqrt{\sum u^2}.$$ (5)

The relative expanded uncertainty (U) was calculated using equation (6) at a 95 % confidence level ($k = 2$)

$$U = u_\text{c} \cdot k \cdot 100.$$ (6)

5. VALIDATION CHARACTERISTICS EVALUATION

Method validation is required to confirm that the analytical procedure is suitable for its intended purpose. Typical steps for a method validation include selectivity, linearity, detection and quantification limits, trueness, precision, etc.

The section below details the performed validation criteria (Table 9) based on the Eurachem Guides [12] and the IUPAC

Table 6. Youden–Steiner experimental design for the analysis of mercury in sediments.

Type	Code	Variable High value X	Variable Low value x	Analysis 1	2	3	4	5	6	7	8
Sample weight (g)	A, a	0.2	0.1	1	2	3	4	5	6	7	8
Flow of oxygen (L/min)	B, b	0.2	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
Mortared	C, c	Yes	No	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.1
Sieved	D, d	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Homogenization	E, e	Yes	No	Yes	Yes	No	No	No	No	Yes	Yes
Setup method	F, f	Fish	Sediment	Fish	Fish	Sed	Sed	Sed	Sed	Fish	Fish
Result				1.61	1.14	1.32	1.02	1.56	1.35	1.83	1.79

Table 7. Ruggedness studies of mercury analyses in sediment samples according to Youden and Steiner statistical analysis.

Variable condition		Results		Difference	Comparison	Acceptability Criteria
High value X	Low value x	Mean X	Mean x	$\Delta\,(X-x)$	Sensitive	
A	a	1.27	1.63	0.36	Non-variable sensitive	
B	b	1.41	1.49	0.08	Non-variable sensitive	
C	c	1.58	1.32	0.26	Non-variable sensitive	$\Delta\,(X-x) < 0.41$
D	d	1.59	1.31	0.28	Non-variable sensitive	
E	e	1.59	1.31	0.29	Non-variable sensitive	

Table 8. Combined and relative expanded uncertainty calculations

Source of Uncertainty	Type	Measured Value	Standard uncertainty $u(x)$	Unit	Relative standard uncertainty $u(x)/x$
Stove heating (u_w)	B	5.00×10^1	8.00×10^{-2}	°C	1.60×10^{-3}
Tare weight (u_w)	B	2.00×10^{-4}	4.83×10^{-8}	kg	2.41×10^{-4}
Concentration of the calibration standard	B	9.37×10^5	4.00×10^0	µg/kg	4.27×10^{-6}
Calibration curve (u_{cal})	B	1.53×10^{-3}	7.78×10^{-19}	µg	5.08×10^{-16}
Repeatability (u_{rep})	A	8.47×10^2	1.30×10^1	µg/kg	1.55×10^{-2}
Intermediate Precision (u_{IP})	A	8.30×10^2	2.61×10^1	µg/kg	3.14×10^{-2}
Combined relative standard uncertainty (u_c)					0.03
Expanded U at 95 % confidence interval ($k = 2$)					6 %

guideline [13]. Selectivity is ensured because the method uses a gold trap for capturing the vapour of Hg and uses a specific wavelength of 253.7 nm for the detection and quantification of Hg_T.

The matrix effect was evaluated to determine if there was a difference between slopes using a Student's t-test. There is no significant difference between slopes.

Linearity was assessed by performing the studentized Breusch–Pagan test, detecting heteroskedasticity. A regression analysis was performed to correct the issue of incorrect standard errors, so that interval estimates and hypothesis tests are valid. The limit of detection and quantification was calculated as indicated by IUPAC [19]. The result for LOD was 27 µg/kg, and LOQ was 83 µg/kg.

Precision was determined by repeatability and intermediate precision. For repeatability, seven replicates at two different concentrations (816 µg/kg and 4853 µg/kg) were evaluated, with results of 2 % of RSD for each concentration, with acceptance criteria of RSD_H 7 % and 10 %.

Intermediate precision was evaluated using a multiple comparisons test and a one-way ANOVA. The p-values were higher than 0.05, which means that there is no significant difference between the variances and means between analysts.

Trueness was assessed as the closeness of the results obtained from the certified value from a CRM using a Student´s t-test. The result obtained was lower than the critical value (2.45), and the bias value was 2.86 µg/kg.

Ruggedness was assessed using a Youden and Steiner statistical analysis to evaluate the extent to which these variations affect the method. None of the variables had a significant effect on the results.

Measurement uncertainty estimation was based on the Eurachem Guide (2012) [11]. This estimation takes into account all the probable experimental errors. The combined relative standard uncertainty (u_c) was 0.03, and the expanded uncertainty with $k = 2$ was 6 %.

The laboratory participated in the Axio AQ665 Round 665 proficiency testing programme, involving 14 laboratories with

Table 9. Fulfil acceptance criteria for all validation characteristics.

Parameter	Acceptance criteria	Result	Fulfil the Acceptance Criteria
Selectivity	Use a specific wavelength to 253.7 nm	Complies	Yes
Matrix effect	Fisher F-test to determine the difference between variances and Student's t-test to determine if there is a significant difference between slopes at a 95 % confidence level.	Complies	Yes
Linearity	Student's t-test to determine if there is a significant linear correlation at a 95 % confidence level.	Complies	Yes
Detection and quantification limits	It was established as three or ten times the standard error of the response between the slope.	Complies	Yes
Repeatability	For two different concentrations was calculated the RSD of the replicates and compare with Horwitz´s relative standard deviation.	Complies	Yes
Intermediate precision	For two different concentrations was calculated the RSD of the replicates. A Levene's test and a one-way ANOVA were performed.	Complies	Yes
Trueness	Seven replicates of the Certified Reference Materials (CRM) were used to calculate the SD and bias. The Student´s t-test was performed.	Complies	Yes
Robustness	Eight experiments were performed and a Youden and Steiner statistical analysis was used.	Complies	Yes
Uncertainty of measurement	The uncertainty measurement of the analysis was based on Eurachem (2012), and the relative expanded uncertainty (U) was calculated at a 95 % confidence interval ($k = 2$).	Complies	Yes

other techniques. The assigned value for Hg was 530 µg/kg, while the laboratory reported a result of 529 µg/kg (*z-score* = - 0.02). This result falls within the acceptable range of 417 µg/kg to 643 µg/kg, indicating satisfactory performance.

6. CONCLUSIONS

The analytical method validated in this study for the determination of Hg_T in sediment samples using the Nippon MA-3000 Direct Mercury Analyzer proved to be reliable, sensitive, and robust. The method eliminates the need for extensive chemical pretreatment, offering a fast and environmentally friendly alternative for Hg quantification in solid matrices.

The method demonstrated excellent selectivity due to the use of a gold amalgamation trap and detection at a Hg specific wavelength (253.7 nm), which minimized interference. Linearity was confirmed across a wide dynamic range (83–4855 µg/kg) with an R^2 value of 0.999, while LOD and LOQ were determined as 27 µg/kg and 83 µg/kg, respectively, indicating high sensitivity.

Repeatability and intermediate precision met the acceptance criteria. Trueness, evaluated using CRM, showed negligible bias. No significant matrix effects were observed, and method robustness was verified under varied analytical conditions, using the Youden and Steiner approach. The relative expanded uncertainty (U) was calculated at a 95 % confidence level ($k = 2$) with a value of 6 %.

Furthermore, the successful performance in a proficiency test reinforces the method's applicability for routine monitoring and regulatory compliance. The results support its use for environmental studies assessing Hg contamination, particularly in regions affected by anthropogenic activities, such as mining or industrial discharge.

In summary, the validated method is well-suited for the accurate and precise determination of Hg_T in sediment samples, fulfilling all criteria for analytical performance. It offers significant advantages in terms of operational simplicity, analytical reliability, and environmental safety, making it a valuable tool for laboratories involved in environmental assessment and public health protection.

ACKNOWLEDGEMENT

REFERENCES

[1] S. Liu, X. Wang, G. Guo, Z. Yan, Status and Environmental Management of Soil Mercury Pollution in China: A Review, J Environ Manage 277 (2021), p. 111442.
DOI: 10.1016/j.jenvman.2020.111442

[2] Y. S. Wu, A. I. Osman, M. Hosny, A. M. Elgarahy, A. S. Eltaweil, (+ 8 more authors), The Toxicity of Mercury and Its Chemical Compounds: Molecular Mechanisms and Environmental and Human Health Implications: A Comprehensive Review, ACS Omega 9 (2024) 5, pp. 5100–5126.
DOI: 10.1021/acsomega.3c07047

[3] P. Almeida Rodrigues, R. Gomes Ferrari, L. Neves dos Santos, C. A. Conte Junior, Mercury in Aquatic Fauna Contamination: A Systematic Review on Its Dynamics and Potential Health Risks, Journal of Environmental Sciences 84 (2019), pp. 205–218.
DOI: 10.1016/j.jes.2019.02.018

[4] U.S. EPA, Indicators: Sediment Mercury. Online [Accessed 28 July 2025]
https://www.epa.gov/national-aquatic-resource-surveys/indicators-sediment-mercury

[5] United Nations, Minamata Convention on Mercury: Text and Annexes, September 2019. Online [Accessed 18 November 2025]
https://minamataconvention.org/en

[6] J. Y. Park, J. H. Won, T. G. Lee, Mercury Analysis of Various Types of Coal Using Acid Extraction and Pyrolysis Methods, Energy and Fuels 20 (2006) 6, pp. 2413–2416.
DOI: 10.1021/EF060209J

[7] J. Wang, Y. Man, R. Yin, X. Feng, Isotopic and Spectroscopic Investigation of Mercury Accumulation in *Houttuynia cordata* Colonizing Historically Contaminated Soil. Environ. Sci. Technol., 56 (2022) 12, pp. 7997–8007.
DOI: 10.1021/acs.est.2c00909

[8] N. Bloom, On the Chemical Form of Mercury in Edible Fish and Marine Invertebrate Tissue, Canadian Journal of Fisheries and Aquatic Sciences 49 (1992) 5, pp. 1010–1017.
DOI: 10.1139/F92-113

[9] S. V. A. Dantas, C. S. A. Felix, F. A. S. Cunha, J. B. de Andrade, S. L. C. Ferreira, Risk Assessment of Mercury in Soil, Water, and Sediments Collected in a Region Impacted by Gold Mining., ACS Omega 10 (2025) 8, pp. 8152–8159.
DOI: 10.1021/acsomega.4c09617

[10] U.S. EPA, Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry, Washington, DC, 1998. Online [Accessed 08 June 2025]
https://www.epa.gov/sites/default/files/2015-07/documents/epa-7473.pdf

[11] S. L. R. Ellison, A. Williams (editors), Quantifying Uncertainty in Analytical Measurement, 3rd edition, 2012. Online [Accessed 06 June 2025]
https://www.eurachem.org/images/stories/Guides/pdf/QUAM2012_P1_ES.pdf

[12] H. Cantwell (editor), Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics. 3rd edition, 2025. Online [Accessed 08 August 2025]
https://eurachem.org/index.php/publications/guides/mv

[13] M. Thompson, S. L. R. Ellison, R. Wood, Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report), Pure Appl. Chem. 74 (2002) 5, pp. 835–855.
DOI: 10.1351/pac200274050835

[14] J. Vessman, R. I. Stefan, J. F. Van Staden, K. Danzer, W. Lindner, D. T. Burns, A. Fajgelj, H. Müller, Selectivity in Analytical Chemistry (IUPAC Recommendations 2001), Pure and Applied Chemistry 73 (2001) 8, pp. 1381–1386.
DOI: 10.1351/PAC20017308138

[15] NIST/SEMATECH. 1.3.5.9. F-Test for Equality of Two Variances, e-Handbook of Statistical Methods, 2012.
DOI: 10.18434/M32189

[16] T. K. Kim, T Test as a Parametric Statistic, Korean J Anesthesiol 68-6 (2015), pp. 540–546.
DOI: 10.4097/KJAE.2015.68.6.540

[17] K. Liao, E. S. Park, J. Zhang, L. Cheng, D. Ji, Q. Ying, J. Z. Yu, A Multiple Linear Regression Model with Multiplicative Log-Normal Error Term for Atmospheric Concentration Data, Science of The Total Environment 767 (2021), p. 144282.
DOI: 10.1016/J.SCITOTENV.2020.144282

[18] C. Yobero, Methods for Detecting and Resolving Heteroskedasticity: An R Tutorial. Online [Accessed 06 June 2025]
https://rpubs.com/cyobero/187387

[19] IUPAC, Detection and quantification capabilities (Section 18.4.3.7), Compendium of Analytical Nomenclature, 3rd ed. 2002.

https://media.iupac.org/publications/analytical_compendium/Cha18sec437.pdf

[20] NIST/SEMATECH. 1.3.5.14. Anderson-Darling Test, e-Handbook of Statistical Methods, 2012.
DOI: 10.18434/M32189

[21] NIST/SEMATECH, 1.3.5.10. Levene Test for Equality of Variances, e-Handbook of Statistical Methods, 2012.
DOI: 10.18434/M32189

[22] NIST/SEMATECH, 3.2.3.1. One-Way ANOVA, e-Handbook of Statistical Methods, 2012.
DOI: 10.18434/M32189

[23] B. Duffau, F. Rojas, I. Guerrero, L. Rodríguez, M. Soto, M. Aguilera, S. Sandoval, Validación de métodos y determinación de la incertidumbre de la medición, Aspectos generales sobre la validación de métodos, Chile, 2010, pp. 28-48. [In Spanish]

[24] E. Karageorgou, V. Samanidou, Youden test application in robustness assays during method validation, Journal of Chromatography A, 1353 (2014), pp. 131–139.
DOI: 10.1016/J.CHROMA.2014.01.050