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# Determination of Total Merccure and Methylmercury in Mangrove Oysters (*Crassostrea gasar*) from a Direct Mercury Analyzer (Dma-80)

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## Abstract

Climate change is a global problem of weather variations characterized by extreme conditions measured over several decades. This paper analyses the impact of climate change on the coastal zone of Nigeria with a major focus on the Niger Delta. It is aimed at ascertaining areas which are most vulnerable to the climatic variables and hazards and proffer mitigations and adaptation strategies. Obviously, coastal erosion, heavy rainfall, flooding, shoreline retreat and coastal submergence, degeneration of mangrove vegetation, seawater intrusion into coastal aquifers, change in ocean dynamics, among others are identified as products of climate change in the coastal zone. However, limited mitigation measures by government and private organisations noted include localized embankment of shoreline, beach nourishment, re-afforestation, channelization, etc. were more of reactive than proactive which pose serious challenges to management of climate change impact on the coastal zone. Holistic attitudinal change by individuals towards environmental protection, promulgation and implementation of eco-friendly policies by government and private institutions, environmental education as a teaching subject from primary schools, capacity development and poverty eradication, etc., are advanced as adaptation strategies to impact of climate change in Nigerian Coastal Zone.

**Keywords :** Methyl mercury; *Crassostrea gasar;* Analytical method

## Introduction

Mercury (Hg) is a chemical pollutant that persists in the environment, especially in its organic form including methylmercury (MeHg). Methylmercury is a neurotoxin produced in aquatic environments in the presence of divalent mercury ($Hg^{2+}$) which undergoes methylation or in the presence of sulfates and iron-reducing bacteria [1]. Indeed, metylmercury (MeHg) is recognized as an environmental pollution problem and represents a danger to human health [2]. It has the ability to penetrate biological membranes, once it enters the body, it is efficiently accumulated and transferred to organisms at higher trophic levels [3]. Methylmercury (MeHg) levels are very high in muscle tissue of top predatory fish ranging from 80% to 90% [4]. The majority of the population is exposed to mercury (Hg) through the consumption of aquatic organisms, mainly fish and seafood [5]. Specifically, large predatory fish that are at the top of the food chain, such as swordfish and tuna, contain high levels of methylmercury (MeHg) and represent significant sources of human exposure to this contaminant. United States Food and Drug Administration [6] and the European Union [7], have published articles to provide health guidelines and warn the general public about the consumption of fish containing high levels of MeHg. The most frequently applied analytical techniques for Hg speciation analysis involve GC (Gas Chromatography), GC - ICP-MS (Gas Chromatography - Inductively Coupled Plasma Mass Spectrometry), Supercritical Fluid Chromatography (SFC), ion chromatography (IC), HPLC - CVAAS (High Performance Liquid Chromatography - Cold Vapor Atomic Absorption Spectrometry) or ICP-MS systems. Several authors have recommended back-extraction of mercury species from solvents for aqueous solutions of cysteine or sodium thiosulfate [8]. Because methylmercury (MeHg) is the most abundant organomercury compound in materials [9].

Direct and automated mercury analyzers are valuable tools for the direct analysis of total mercury (THg) in a variety of biota matrices [10-12] and is widely used in laboratories which analyze total mercury (THg), in fishery products, organic mercury can be considered as MeHg, with negligible error. Therefore, proper extraction of organic mercury and then analyzed with a direct mercury analyzer can potentially be used for mercury speciation. Such an application has already been reported [13-16], the European Commission published a standard operating procedure based on AMA-254 or DMA- 80 for the determination of MeHg [17,18] and similar work was recently carried out by Sabine [19] The aim of this study was to determine metylmercury in mangrove oysters (*Crassostrea gasaret*) using the methods proposed by the European Commission and by Sabine, consisting of an extraction of MeHg, and a determination with a direct mercury analyzer (DMA-80):

To evaluate the method on a wide spectrum of MeHg levels, we used the following Certified Reference Materials (CRMs): IAEA-436 (tuna muscle tissue), IAEA-407 (trace metals and methylmercury in fish homogenate) and IAEA -461 (trace element in clam).

## Materials and Methods

### Equipment

**DMA-80 direct mercury analyzer :** The mercury analyzer (DMA-80) works according to the principle of AAS (Atomic Absorption Spectrophotometer). It is based on the absorption of a specific wavelength (λmax = 253.7 nm) by the analyte. The DMA-80 analysis

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system consists of 4 units:

-A gas unit : oxygen cylinder, it can be replaced by an air compressor.

-A unit of analysis: DMA-80 (tricell).

-A data processing and observation unit : a computer.

-A reject unit : the Hg trap tube.

**Chemical material :** The water used for the preparation of all solutions is ultra-pure water, the Reference Materials (CRMs) applied as test samples in the method validation process are as follows: IAEA-436 (tuna fish homogenate), IAEA-407 (trace metals and methylmercury in fish homogenate) and IAEA-461 (trace element in clam).

Hydrochloric acid (HCl) (25%, Suprapur) or hydrobromic acid (HBr) (47%, pro analysis), both from Merck, Darmstadt, Germany were used for hydrolysis of the studied samples. A 0.002 M sodium thiosulfate solution (Suprapur, Merck) or 1% (w/v) L cysteine (SigmaAldrich, Steinheim, Germany) prepared in 12% (w/v) anhydrous sodium sulfate and 0.8% (w/v) sodium acetate (Suprapur, Merck) were used for the reto-extraction.

A 1000 mg/kg inorganic mercury standard solution at 12% (v/v) nitric acid (Trace Cert, Fluka, Steinheim, Germany). The working solutions were prepared by diluting the standard solutions at 1% (v/v) nitric acid (40%, Suprapur Merck), 0.1% (v/v) hydrochloric acid HCl (Suprapur, Merck) and 0.2% potassium dichromate (analytical grade, 10% (w/v), Merck.

**Species studied :** In this study we applied this method to an aquatic species, mangrove oysters (*Crassostrea gasar*): The mangrove oyster (*Crassostrea gasar*) is a bivalve, sessile mollusk found in mangrove areas, either attached to the roots of these trees or attached to plant debris or other supports. It is a filter feeder. It feeds on living organisms (plankton), detrital organic matter (tripton) and inorganic particles (seston, mud, fine sands, shell debris). The gills and cilia play an important role in the way it is fed [20]. The choice of *Crassostrea gasar* is significant in more ways than one:

- It is consumed a lot and constitutes a significant source of income for lagoon populations of the Ivory Coast,

- It meets most of the criteria for choosing pollution indicator organisms defined by Barbaro et al. [21].

Indeed, *Crassostrea gasar* is available in all seasons, abundant, easy to sample and restricted mobility. In addition, it is likely to bioaccumulate pollutants at high rates.

## Method

**DMA-80 operating mode :** The analyzes were carried out using a direct mercury analyzer (DMA-80, Millestone). The sample (solid or liquid) is dried at 220°C then thermally decomposed at 725 C. The gaseous decomposition products are transported in a stream of oxygen through the catalytic section of the oven, where the catalyst allows complete oxidation, halogens and oxides of nitrogen, sulfur are trapped. Subsequently, the different species of mercury are converted into elemental mercury vapor (Hg0) and selectively trapped on a gold-based amalgamator. After flushing the system with oxygen, the amalgamator heats up quickly, releasing mercury vapor. The flow of oxygen carries mercury vapor to the absorbance cell on the light path of a single wavelength atomic absorption spectrophotometer, a low pressure mercury vapor lamp is used at the wavelength of 253.7 nm. The detector is connected to a computer for data acquisition and analysis. The temperatures for drying and the decomposition step, were set by default at 220°C and 725°C, respectively. The drying time (s) have been programmed to 0.7 times the volume (lL) of the sample injected. Decomposition and waiting time were 150 s and 45 s, respectively.

**Methyl mercury extraction method**

**Method 1 :** 0.1 to 0.8 g of the sample is taken. Place the sample in a 50 mL polypropylene centrifuge tube, add 5 mL of hydrochloric acid (HCl). 25% (v/v) The mixture is shaken vigorously for 30 seconds. Next, 10 ml of toluene was added to the tube and a vortexing method was then applied for 3 minutes to ensure phase homogenization. Centrifuge the mixture at 5,000 rpm for 5 to 20 min. A known volume (4 to 8 ml) of the upper organic phase is removed and transferred to a second 50 ml polypropylene centrifuge tube containing 5 to 10 ml of 0.002 M sodium thiosulfate solution. This second tube is shaken vigorously ( vortex, 3 min) and centrifuged at 5000 rpm for 5 to 15 min. Two ml of the lower aqueous phase, which contains the extracted organic mercury, is transferred to a 15 ml polypropylene container or glass vial, using a glass Pasteur pipette. Then, an aliquot (50-400 l L) of the extract is analyzed directly with DMA-80 The thiosulfate extract is judged to be stable for 2 days at a temperature of 4 C. [19]

**Method 2 :** This method involves taking 0.1 to 0.5 g of sample, which is placed in a 50 mL polypropylene tube. Then 10 ml of hydrobromic acid (HBr) is added to hydrolyze the sample followed by addition of 20 ml of toluene. The mixture should be homogenized for 2 minutes and centrifuged for 10 min at 3000 rpm. After centrifugation, the organic phase is transferred to a tube containing 6.0 mL of 1% cysteine solution. A second organic extraction is carried out. A 0.5 ml aliquot of the cysteine extract is then analyzed with DMA-80. This procedure has been proposed by the European Commission as a Standard Operating Procedure (SOP) for the determination of MeHg by the direct mercury analyzer in seafood to all European reference laboratories for trace elements in food. and animal feed [18].

## Results and Discussion

The direct tricel mercury analyzer (DMA-80), provides three working ranges for the detection of mercury: (0-10), (10-20) and (20-1500) ng. Each range is independently calibrated for best results. The direct mercury analyzer was calibrated with the standards of 0.0, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ l of mercury for the lower cell. Concentration (0-10 ng) and 0.0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mg/ l for high concentration cells (10-1500 ng. The limits of quantification (LQM) and detection (LDM) were 0.77 and 0.25 ng/g, respectively. The LQM was established by the lowest calibration point; the LDM was 3.08 times higher low than the LQM when the signal-to-noise ratio was greater than 10, the repeatability was determined at 0.8 ng/g, the replicability at 2.97 ng/g with respectively coefficients of variation of 2.34% and 1, 9%, trueness at 4% and accuracy at 96% with a coefficient of variation of 1.9%.

The humidity rate and the lipid content were determined, for the reference materials we obtained a humidity rate ranging from 1.07% to 9.04% and the rate of lipid matter varied from 37.24% to 62 , 51%, for the oysters the moisture content varied from 12.37% to 14.62% and the fat content varied from 5.2% to 13.26%. These results clearly show that oysters contain less fat.

The methylmercury concentration (MeHg) for the certified reference materials (CRM) IAEA-461, IAEA-436 and IAEA-407, are shown in Table 1. Analysis of the certified reference materials showed recoveries of 95% to 98% with a variation coefficient of 2 to 4%. The

results are in conformity with those of the certified values, thus showing that the methods proposed for the extraction of methyl mercury (MeHg) as a procedure for measuring this form of mercury (Hg) in fishery products are procedures which are well suited. The extraction procedures used in this study were then compared with each other, the procedure of Sabine et al. [19] and the standard operation procedure for methyl mercury proposed by the European Union [18]. The results obtained are presented in Table 1. To evaluate the variability of the method, three extractions were carried out for each reference material, these results clearly show that the extractions were made under good conditions with acceptable deviations, which vary. from 0.002 to 0.04 µg/kg. During the extractions the effects of the concentration of hydrochloric acid (HCl) and hydrobromic acid (HBr) on the extraction recovery of methylmercury was evaluated. The use of 25% (v/v) hydrochloric acid(HCl) and 47% (y/v) hydrobromic acid (HBr) gave good recovery of about 97.98% on average and when 'we increased the concentration of hydrochloric acid we observed a decrease in recovery probably due to the degradation of methyl mercury as reported by Sabine et al. [19]. A similar case has been observed with nitric acid and sulfuric acid [7]. In addition, the effects of solvent extraction time were also investigated. Overall, the extraction conditions were set at 25% (v / v) for hydrochloric acid (HCl) and 47% (v / v) for hydrobromic acid (HBr) and 3 min. stirring, for the solvent extraction steps and 10 to 20 minutes of centrifugation. Ultraviolet (UV) and visible (VIS) radiation lead to the degradation of methyl mercury [22]. In this regard, parallel extractions, under and without UV exposure, were performed. No significant difference in the recovery of methylmercury (MeHg) was observed. Although some published protocols recommend a double solvent extraction [18,14], the amounts obtained for the reference materials used using these two methods show that a single extraction is sufficient. In order to avoid long centrifugation times, the sample size and the volume of toluene were kept as small as possible, but sufficient to obtain a measurable signal at DMA-80. The mass fraction of methylmercury (MeHg) produced by these two methodologies shows results in good agreement between them. One of the advantages of the extraction protocols used is the significant reduction in the volume of organic solvent was.

The total mercury concentrations are significant and a fraction has turned into methyl mercury.

The oyster analysis results are shown in Table 2, these results clearly indicate that the mangrove oysters from Boulay Island (Cote d'Ivoire) contain methylmercury. The significant correlation observed (Figure 1) between the methylmercury concentrations and the weight of the specimens means that the accumulation of methylmercury is proportional. to the weight of individuals and consequently, it is the large individuals which present the highest contents in their flesh. This indicates a difference in filtration capacity between small and large individuals, and an accumulation of methylmercury over time from ingested food [23,24]. The concentrations of this chemical pollutant are high with a minimum concentration of 0.46 mg / kg and a maximum concentration of 2.01 mg/kg, the average concentration of which is 1.005 mg/kg (Figure 2). Indeed, the oyster is a filter feeder and planktonophagous mollusc. Its diet and filtration capacity depend on the physiological state of the mollusc and its stage of development (larva, juvenile, adult) [20]. The larger the individual, the greater its filtration capacity and the more metallic elements it concentrates via food. This correlation between the concentrations of methylmercury and the weight of marine and lagoon organisms has already been reported in the literature. However, as Cossa et al. [20-29].

| | L-cysteine extract | | | Sodium thiosulfate extract | | |
|---|---|---|---|---|---|---|
| **Number of extracts** | **IAEA-436** | **IAEA-407** | **IAEA-461** | **IAEA-436** | **IAEA-407** | **IAEA-461** |
| Number of extractsConcentration in MeHg of extract 1 (mg / kg) | 3.23 | 0.27 | 0.06 | 3.10 | 0.28 | 0.06 |
| Number of extractsConcentration in MeHg of extract 2 (mg / kg) | 2.76 | 0.22 | 0.05 | 3.26 | 0.23 | 0.06 |
| Number of extractsConcentration in MeHg of extract 3 (mg / kg) | 2.40 | 0.22 | 0.06 | 3.38 | 0.27 | 0.05 |
| Certified value (mg / kg) | 3.62 | 0.2 | 0.0623 | 3.62 | 0.2 | 0.0623 |
| Coefficient of variation | 4.02% | 3.16% | 2.7% | 2.01% | 2.4% | 2.3% |
| Recovery | 95.96% | 97.98 | 96.99 | 97.96% | 98.94% | 98.99% |

**Table 1:** Comparison of the procedures used.

| Sample | Absorbance $Hg_T$ | $Hg_T$ content (ng) | $Hg_T$ concentration (mg / kg) | Absorbance MeHg | MeHg content (ng) | MeHg Concentration (mg/kg) |
|---|---|---|---|---|---|---|
| Oyster 1 | 0.3041 | 11.5547 | 3.3617 | 0.0603 | 2.12 | $0.6666^{+}_{-}$ 0.017 |
| Oyster 2 | 0.1739 | 7.2985 | 2.2056 | 0.1184 | 4.9692 | $1.5017^{+}_{-}$ 0.022 |
| Oyster 3 | 0.3252 | 13.3811 | 4.0634 | 0.1044 | 4.2958 | $1.3045^{+}_{-}$ 0.015 |
| Oyster 4 | 0.2985 | 13.6974 | 3.9137 | 0.1537 | 7.0529 | $2.0140^{+}_{-}$ 0.024 |
| Oyster 5 | 0.2013 | 8.4360 | 2.3921 | 0.1151 | 4.8236 | $1.3678^{+}_{-}$ 0.009 |
| Oyster 6 | 0.2215 | 8.2195 | 2.3699 | 0.0433 | 1.6068 | $0.4633^{+}_{-}$ 0.017 |
| Oyster 7 | 0.2298 | 26.0835 | 7.6753 | 0.0170 | 1.9298 | $0.5678^{+}_{-}$ 0.016 |
| Oyster 8 | 0.1854 | 7.1269 | 2.2399 | 0.0685 | 2.6332 | $0.8276^{+}_{-}$ 0.019 |
| Oyster 9 | 0.2377 | 13.7260 | 2.6752 | 0.0412 | 2.3791 | $0.4637^{+}_{-}$ 0.020 |
| Oyster 10 | 0.3178 | 12.1552 | 4.2783 | 0.0649 | 2.4823 | $0.37^{+}_{-}$ 0.011 |
| Medium | - | 11.3159 | 3.5177 | - | 3.4463 | $1.0050^{+}_{-}$ 0.017 |

**Table 2 :** Concentration du méthylmercure dans les huitres de mangroves (*Crassostrea gasar*).


**Figure 1:** Correlations between the HgT concentrations of *Crassostrea gasar* and their mass.

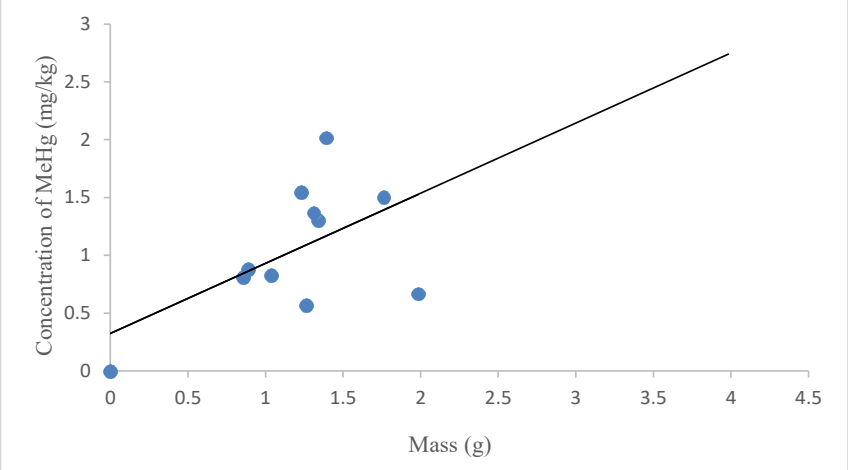


**Figure 2:** Correlations between the MeHg concentrations of *Crassostrea gasar* and their mass.

## Conclusion

At the end of this work, we note that the determination of methyl mercury was carried out in accordance with the procedures proposed by the European Union and Sabine et al., These procedures consisted in extracting the organic mercury with a solvent and then carrying out a retro - aqueous phase extraction followed by detection with a direct mercury analyzer (DMA-80). Important parameters influencing the procedure such as acid, extraction solvent and minimum time have been optimized. Analysis of the oysters indicated significant concentrations of methylmercury. Overall, the proposed method is simple, cost effective and allows the analysis of a large number of samples per day. During the extraction stages, the volume of waste produced is considerably reduced. In light of the results presented in ultimately be a suitable method for the determination of total mercury and methylmercury.

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