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Baseline study for the total mercury determination in Yemeni fish

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ABSTRACT

The high levels of mercury toxicity in humans make it necessary to monitor mercury levels in food, pharmaceuticals, and the environment to minimize human exposure. Between June 2020 and October 2021, researchers collected 240 fish samples from different locations along the Yemeni coast to evaluate mercury contamination. The Direct Mercury Analyzer was used to determine the concentration of mercury in each sample. To ensure method accuracy, a series of triplicate mercury concentration analyses were conducted. The samples ranged from 2 to 100 ng to determine linearity and repeatability i.e., within-day variation. The results showed a high level of precision, with a correlation coefficient of 0.9990 and a repeatability of 1.34 %–5.62 % RSD range. The method was also highly accurate, as the mercury recovery results from the contaminated fish samples ranged from 96.77 % to 105.14 %. The limits of detection and quantitation of mercury were 0.0015 ppm and 0.0049 ppm, respectively. This allowed the method to detect trace amounts of mercury in fish meat. Mercury concentration in the 240 fish samples did not exceed the FDA, but below the 0.5 ppm specified limit of YSMO.

1. Introduction

Mercury is a global contaminant and a very hazardous element due to its accumulative and persistent nature in the environment and living organisms [1,2]. Mercury, on the other hand, is of great interest since it is widely utilized in industry for the manufacturing of chemicals, insecticides, electrical apparatus, paints, amalgam tooth fillings, and so on [3]. As a result, mercury concentrations vary across the environment, including air, water, soil, and living organisms [4]. The relative toxicity of mercury is determined by its chemical form, and for methylmercury being one of the most poisonous compounds causing irreversible damage to the nervous system [5,6].

Human exposure to mono methylmercury (MMHg), the predominant form of mercury in fish due to biomagnification in the marine food chain, is primarily through fish intake [6,7]. The presence of mercury in fish can be especially dangerous for pregnant women, nursing mothers, and small children [4]. Several investigations have shown that organic mercury can enter the placenta and damage the fetus by disrupting the blood-brain barrier [8].

Mercury exposure causes a variety of symptoms, including impaired vision and hearing, dizziness, vomiting, headache, muscle

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weakness, allergies, a weakened immune, brain damage, and death [9]. Mercury and heavy metal bioaccumulation in fish muscle is determined by various characteristics such as size, age, sex, lipid content, and the depth of the fish habitat, making its measurement problematic in environmental studies [10–12].

Mercury was determined using various analytical techniques such as UV vis spectrometry [13], inductively coupled plasma mass spectrometry (ICP-MS) [14,15], inductively coupled plasma atomic emission spectrometry (ICP-AES) [4,16], neutron activation analysis (NAA) [17], and cold vapor atomic absorption spectrometry (CVAAS), with the latter being the most commonly used for measuring mercury in fish tissue [18]. Some of these methods are either too expensive such as ICP-MS or require tedious sample pretreatment to detect trace levels of Hg in biological matrices such as GC and CV- AAS.

Literature studies revealed data about mercury in fish samples. In Persian Gluf, mercury in parrotfish (Scarus ghobban) was 0.07254 ± 0.0020 ppm using FAAS [19]. Determination of mercury in pompano (Trachinotus carolinus) and palometa (Trachinotus goodei) samples in Brazil [20]. In Vietnam, mercury in Whipfin silver biddy (Gerres filamentosus Cuv.) and Flathead grey mullet (Mugil cephalus L.) was 0.097 ppm using cold vapor atomic absorption spectrometry [21].

In California, mercury of 0.14 ppm in dolphinfish (Coryphaena hippurus) was observed [22]. In California (2020), mercury concentration in Haemulopsis elongates and Pomadasys macracanthu samples were 3.748 ppm and 0.574 ppm using CV-AAS [23]. Maryam Ravanbakhsh et al., 2020 respectively found mercury of 3.15 ± 0.474 and 0.486 ± 0.116 ppm in Johnius Belangerii (C) and Cynoglossus Arel in Persian Gluf [24]. In North Sulawesi, mercury in Yellowfin tuna (Thunnus albacares) and Indian mackerel (Rastrelliger kanagurta) were 0.6754 ppm and 0.73784 ppm using MA-3000 [25]. In Arabian Gulf, Nuray Alizada et al., 2020, found Hg of 0.04–0.18 ppm in Indian anchovy (Stolephorus indicus) using Varian SpectrAA 220 FS [26]. In California, mercury in striped marlin Kajikia audax and blue marlin Makaira nigricans samples was measured [27]. In France,(Antoine Minet et al., 2022), studied mercury in cuttlefish Sepia officinalis [28]. In Thailand, DMA was used to determine mercury in Barracuda (Sphyraena putnamae) and found to be 0.00958 -0.314 ppm [29].

Direct mercury analyzers, such as the DMA-80, have recently acquired favor for total Hg measurement because of their ability to successfully analyze mercury in both liquid and solid matrices, high sample throughput, and comparatively low detection limits and cost [30–32]. The DMA-80 analysis of Hg involves thermal decomposition followed by gold amalgamation and detection using atomic absorption spectrometry. Moreover, DMA-80 does not require a sample preparation step prior to mercury determination, which saves time and money.

The Republic of Yemen, which occupies the southeastern to southernmost tip of the Arabian Peninsula, has a coastline that stretches over 2500 km. These extensive coastal waters are bordered by two bodies of water with vastly distinct marine ecosystems: the Red Sea and the Gulf of Aden. The Red Sea is a mild and salty body of water, whereas the Gulf of Aden is cooler and less salty. This variation in environmental conditions enables a great diversity of fish species to flourish, with a recent report indicating that over 733 species of fish inhabit just the eastern part of the Gulf of Aden (the Socotra Archipelago) [33].

A survey of the scientific literature revealed a scarcity of studies on the mercury contamination of Yemeni fish species. The data in these publications is limited in terms of fish species analyzed and fishing sites covered, prompting more comprehensive research [7]. Thus, the current study's goal is to conduct a more comprehensive study to determine total mercury concentrations in Yemeni fish, in which a large number of fishing sites on both the Red Sea and the Gulf of Aden are surveyed and more fish species are analyzed, to provide a preliminary database of mercury pollution in Yemeni fish.

2. Materials and methods

2.1. Reagents and materials

The mercury standard solution 1000 mg/L in 10 % HNO_3 was purchased from PerkinElmer, USA. Hydrochloric acid 37 % w/w (extra pure reagent grade, ACS) was from Aldrich, Spain. Deionized water was produced in the lab with a resistivity of 18.2 $\mu\Omega$.cm using Direct Q3-Millipore - USA.

2.2. Instrument

The Dual-cell Direct Mercury Analyzer DMA-80 (Milestone, Waltham, Sorisole, Italy) fish sample analyzer, which is based on the theory of atomic absorption spectrometry, was used to determine the total mercury amounts in fish samples. A silicon UV photode-tector and a dual spectrophotometer cell are included with the DMA-80. The combustion and carrier gas were produced using a high-purity air compressor located in Milestone, Italy. To prevent contamination, nickel sample boats were pre-cleaned by rinsing them with deionized water, dried, and then heated in a furnace for 2 min to 650 °C. Following US-EPA Method 7473 [34], the DMA-80 analytical technique and settings were used.

2.3. Preparation of mercury standard solutions

From the stock solution of 1000 ppm mercury, intermediate standard solutions of 1 and 10 ppm were made. The intermediate solutions were diluted appropriately to create working standard solutions of 0.01–0.2 ppm. A 2 % HCl solution was used to stabilize each mercury standard solution.



Fig. 1. Republic of Yemen Map showing fish sampling sites.

2.4. Sampling sites, Sample collection and preparation

The samples were taken from Yemen's different fishing ports on the Arabian Sea and Red Sea coasts. The study protocol was approved by the animal ethics committee of Biological Science, Sana'a University (ethical code: BAHSS102). Sampling locations are given in Fig. 1. Every sample was collected between June 2020 and October 2021. The present investigation adhered to the guidelines of sampling, sample preservation, and transportation established by the Minamata Convention and other reputable organizations, including the WHO and EPA-US [35]. The time, place, and species of the fish samples collections were noted down. Additionally, the length of each fish was measured, and the fish samples were then placed in polyethylene bags and kept in a deep freezer for the use. The fish samples were sent straight to the lab and washed with tap water to ensure cleanliness.

Fish tissue samples were chopped into tiny bits using a knife, and then pulverized to create a smooth, uniform paste. For analysis, 0.2 g was weighed straight into the nickel boat using an analytical balance. The sample was dried at 200 °C for 90 s inside the DMA-80. After that, the temperature in the furnace was raised to 650 °C and left for 120 s, allowing the sample to break down. The mercury vapors were then delivered to the catalyst tube, where all forms of mercury in the sample were converted to elemental mercury. The elemental mercury was then conveyed as vapor to the gold amalgamator, where it was quantitatively trapped, by the carrier gas at a flow rate of 120 mL min⁻¹. The atomic absorption spectrophotometer was used to detect the absorption intensity at a wavelength of 253.65 nm after a mercury particle was released into it. Finally, the DMA-80 was flushed through, and the mean of three consecutive readings of an empty sample boat was taken prior to each batch of sample analysis to ensure boat cleanness [34]. The Easy Doc program integrated the resulting mercury peak height (Milestone Inc., Bergamo, Italy), and the total mercury content was reported in ppm.

The 240 fish samples examined in this project belonged to the following 31 fish families: *Gerreidae, Carangidae, Mugilidae, Portunidae, Lutijanidae, Polyodontidae, Coryphaenidae, Sciaenidae, Hemiramphidae, Clupeidae, Scombridae, Synodontidae, Engraulidae, Penaeidae, Haemulidae, Sarranidae, Salmonidae, Lethrinidae, Archarhinidae, Sphyraenidae, Poeciitidae, Ariidae, Morontidae, Serraniidae, Rachycentridae, Noctuidae, Scaridae, Tetraodonidae, Sepidae, Istiophoridae, and Xiphidae. As shown in Table 4.*

2.5. Spiked samples preparation

To verify the accuracy of our analysis method, 0.2 g of homogenized fish sample muscle was placed into a sample boat and spiked with 10, 25, 50, 100, 250, and 500 μ L of a 0.1 ppm standard solution of mercury followed by DMA analysis. The difference between the spiked and unspiked concentrations was used to compute the recovery.

2.6. Validation study

The method of analysis' validation study parameters, which included linearity, precision, accuracy, limits of detection, and quantification, were evaluated.



Fig. 2. Method Calibration curve of Mercury Standards.

Table 1
Precision results of mercury analysis.

Spiked Amount of Hg (ng)	Detector response (Measured Amount of Hg))(A)			Average (A)	SD	%RSD	
	Replicate 1	Replicate 1 Replicate 2 Replicate 3					
2	0.00212	0.0019	0.00197	0.002	0.0001	5.62	
5	0.00498	0.00503	0.00471	0.00491	0.0002	3.45	
10	0.00997	0.01026	0.01	0.01007	0.0002	1.61	
20	0.01951	0.01944	0.02068	0.01987	0.0007	3.50	
40	0.03974	0.03847	0.04101	0.03974	0.0013	3.20	
60	0.05605	0.05411	0.05545	0.0552	0.0010	1.80	
80	0.07646	0.07723	0.07512	0.07627	0.0011	1.40	
100	0.09147	0.09269	0.09394	0.0927	0.0012	1.34	

Table 2

Recoveries for mercury in fish samples.

No.	Hg (ng) in unspiked sample	The volume of 0.1 ppm Hg Std. (μl) added	Added Hg amount (ng)	Analyzed amount (ng), $(n = 3)$	Recovered amount of Hg (ng)	%R	%Bias
1	11.542	10	1	12.5934	1.0514	105.14	5.14
2	11.542	25	2.5	14.1267	2.5847	103.39	3.39
3	11.542	50	5	16.5259	4.9839	99.68	-0.32
4	11.542	100	10	21.6275	10.0855	100.86	0.86
5	11.542	250	25	36.2372	24.6952	98.78	-1.22
6	11.542	500	50	59.9275	48.3855	96.77	-3.23

3. Results and discussion

3.1. Method validation

3.1.1. Linearity

The method linearity was evaluated by the analysis of eight different concentrations of mercury standard. The correlation coefficient (R^2) determination was calculated using the least-square analysis. The method's linearity was evaluated by plotting detector response absorbance (A) versus mercuric spiked concentrations that ranged from 2 to 100 ng as shown in Fig. 2. The correlation coefficient of the method as shown in Fig. 2 reached 0.9990.

3.1.2. Precision (repeatability)

The assessment of the method's precision was conducted by means of an intra-day repeatability analysis. On the same day, actual samples were analyzed in triplicate and the relative standard deviation was computed. The method's RSD ranged between 1.34 % and 5.62 % as shown in Table 1. This was within the acceptable range specified by the AOAC for the peer-verified method [36]. However, M. Augelli (2007) reported % RSD of 11 [37], whereas J. Calderon (2013) reported %RSD less than 12.3 [38].

3.1.3. Accuracy (recovery)

To conduct the accuracy assays, six separate analyses were carried out in triplicate on fish samples spiked with various amounts of mercury standards ranging from 0.1 to 1 ppm. The recovery percentages, as presented in Table 2, varied from 96.77 % to 105.14 %. Additionally, the range of predicted bias values, from 0.32 % to 5.14 %, fell within the permissible percent of RSD range for the peerverified technique as set by the AOAC [36]. These results indicated the high method's accuracy for the total mercury determination in fish samples. These results are in good agreement with that reported by A. Shah (2012) % R (99–100.1) [39], D. Benjamin (2013) % R

Table 3

Fish samples results.

#	English Name	Sciences Name	Family	Length (cm)	No. of Fish	Average (ppm)	Concentration Range
L	Whipfin silver biddy	Gerre filamentosus	GERREIDAE	31–54	2	0.0212	(0.0165–0.0259)
	Arabi	Valamugil seheli	MUGILIDAE	32–38	5	0.0410	(0.02231-0.0674)
	Abumakas	Porturus pelagicus	PORTUNIDAE	22	2	0.0459	(0.0249-0.0668)
	Gambri	Penaeus japonicus	PENAEIDAE	10-15	10	0.0463	(0.0114-0.0998)
	Snappers	Lutjanidae	LUTJANIDAE	41-43	2	0.0475	(0.0220-0.0729)
	Paddlefish	Polyden spathula	POLYODONTIDAE	41-43	2	0.0535	(0.0485-0.0584)
	Bagha	Rastrelliger barchysoma	SCOMBRIDAE	22–34	6	0.0544	(0.0113–0.1136)
	Common dolphin fish	Coryphaena lippurus	CORYPHAENIDAE	69–70	2	0.0627	(0.0551–0.0702)
	Kurdoll	Johnius carultta	SCIAENIDAE	30	2	0.0630	(0.0610-0.0651)
0	Black baned grafish	Hemiramphus far	HEMIRAMPHIDAE	23–30	2	0.0640	(0.0245–0.1034)
L	Sabarii	Megalaspis cordyla	CARANGIDAE	23–34	9	0.0645	(0.0291-0.1271)
2	Rain bow sardine	Dussumieria acuta	CLUPEIDAE	22	2	0.0694	(0.0690-0.0697)
3	Blubberlip snapper	Lutjanw rivulatus	LUTJANIDAE	33–35	2	0.0714	(0.0687–0.0740)
ł	Batabet	Scomber japonicus	SCOMBRIDAE	24–31	2	0.0723	(0.0294–0.1151)
;	Rosy goatfish	Perupeneus rubescens	MUGILIDAE	27–33	5	0.0732	(0.0494–0.1114)
5	Brushtooth lizard	Saurida undosquamis	SYNODONTIDAE	34–38	5	0.0788	(0.0352-0.1012)
,	Indian anchovy	Stolephorus indicus	ENGRAULIDAE	3–4	4	0.0804	(0.0755–0.0857)
3	Indian Mackerel	Rastrelliger kanagurta	SCOMBRIDAE	28–33	3	0.0811	(0.0605–0.0993)
)	Johns snapper	Lutjanus johni	LUTJANIDAE	31–56	4	0.0867	(0.0530 - 0.1403)
)	Salmon	Salmo	SAIMONIDAE	32–39	7	0.0873	(0.0453–0.1356)
	Grey snapper	Lutjanus griseus	LUTJANIDAE	39–45	2	0.0877	(0.0753-0.1001)
	Baiad	Whitefin trevally	CARANGIDAE	26-58	15	0.0894	(0.0353–0.1792)
	Antak	Nempiterus japonicus	LUTJANIDAE	42-45	3	0.0949	(0.0702–0.1294)
	Hadas	Plectrohynchus gaterinus	HAEMULIDAE	32-45	3	0.0991	(0.0644-0.1176)
5	Arabian scad	Decapterus macarellus	CAESIONIDAE	24-31	2	0.1006	(0.0911-0.1101)
5	Gahash	Lethrinselongatus	LETHRINIDAE	29-55	11	0.1027	(0.0502-0.1754)
7	Epinephelus King Cick	Epinphelus tukula	SARRANIDAE	70–75	2	0.1094	(0.1046-0.1141)
3	King fish	Scomberomorus caralla	SCOMBRIDAE	33-39	2 5	0.1129	(0.0831 - 0.1427)
9 D	Derak Walad	Scomberomorus gutatus	SCOMBRIDAE ARCHARHINIDAE	97–160 39–40	5 2	0.1559	(0.0639 - 0.1608)
l	Double spotted	Rhizoprionodomacutus Scomberoides	CARANGIDAE	39–40 52–73	2 4	0.1261 0.1272	(0.0728 - 0.1794) (0.0958 - 0.1562)
2	Kud	Sphraena putnamiae	SPHYRAENIDAE	30-46	6	0.1435	(0.0744-0.2043)
3	Affinis fish	Gambusia affinis	POECIITIDAE	25	2	0.1511	(0.1435–0.1586)
4	Xiphias	Xiphias gladius	NOCTUIDAE	80-95	4	0.1645	(0.1450-0.1829)
5	Dogtooth tuna	Gymnosada unicolor	SCOMBRIDAE	94–98	2	0.1821	(0.1594-0.2049)
5	Palometa	Trachinotus goodei	CARANGIDAE	39-43	2	0.1839	(0.1639-0.2038)
7	Nakim	Plectrohynchus flaromculatus	HAEMULIDAE	36–47	4	0.1908	(0.0969–0.2661)
3	Sakhlah	Rachycentron candum	RACHYCENTRIDAE	95–140	11	0.1973	(0.1053-0.3433)
9	Striped bass fish	Striped bass	MORONIDAE	28–37	4	0.1988	(0.1065-0.2793)
)	Giant catfish	Ariusthalassinus	ARIIDAE	27-42	5	0.201	(0.1063-0.2873)
L	Parrot fish	Chlorurus	SCARIDAE	28-38	6	0.2058	(0.1339-0.2648)
2	Frigate tuna	Auxithazard	SCOMBRIDAE	56–58	2	0.2368	(0.1829-0.2906)
3	Barracudas	Sphyraena obtusata	SPHYRAENIDAE	60–65	2	0.2563	(0.2299-0.2826)
1	Kashar	Cephalispachy centron	SERRANIIDAE	37–119	6	0.02641	(0.1093–0.4598)
5	Harab	Alectis ciliaris	CARANGIDAE	38–93	4	0.2904	(0.1743–0.4494)
5	Logtail tuna	Thunnus tonggol	SCOMBRIDAE	94–96	2	0.3153	(0.3001–0.3305)
7	Thamad	Thunnus abacares	SCOMBRIDAE	143–195	14	0.3604	(0.2891-0.5458)
3	blowfish	White spotted puffer	TETRAODONIDAE	49–78	6	0.3992	(0.3381-0.5298)
9	Cuttlefish	Sepia parshadi	SEPIIDAE	29–38	2	0.4008	(0.3178–0.4837)
)	Wahoo	Acanthocy biumsolandri	SCOMBRIDAE	114	2	0.4600	(0.4520-0.4680)
1	Darob	Scomberoides commersonianus	CARANGIDAE	113–116	2	0.6906	(0.6099–0.7712)
2	Spottail shark	Carchartiinus sorrah	CARANGIDAE	84–127	15	0.7140	(0.4138–1.1860)
3	Sandar shark	Carcharhinus plumbcus	ARCHARHINIDAE	107 - 135	3	0.7159	(0.5998-0.9220)
4	Anbaria	Makaira indica	ISTIOPHORIDAE	178–192	3	0.8053	(0.7045–0.8602)
5	Sword fish	Xiphias gladius	XIPHIDAE	200	2	1.3348	(1.300 - 1.3691)

= 99 % [40], and in (2018) A. Badamchi %R (94–108) [4].

4. Limits of detection (LOD) and limits of quantification (LOQ)

Using the slop (S) and RSD values of the calibration curve shown in Fig. 2, the limit of detection (LOD) and limit of quantitation

(2)

Table 4

Statistical analysis of mean Hg among fish families.

	Arabian	n	Average	Red	n	Average	T cal 95 %
1	MUGILIDAE	7	0.0568	MUGILIDAE	3	0.0578	0.9559
2	LUTJANIDAE	6	0.0825	LUTJANIDAE	7	0.0785	0.8349
3	CARANGIDAE	22	0.1688	CARANGIDAE	29	0.4254	0.0018
4	SCOMBRIDAE	23	0.2627	SCOMBRIDAE	17	0.1713	0.057
5	SPHYRAENIDAE	2	0.1440	SPHYRAENIDAE	6	0.181	0.5409
6	TETRAODONIDAE	2	0.4940	TETRAODONIDAE	4	0.3518	0.1475
7	RACHYCENTRIDAE	3	0.2701	RACHYCENTRIDAE	8	0.17	0.1648



Fig. 3. Mercury concentration of the Yemeni fish samples.

(LOQ) were calculated using the following equations:

$LOD = 3 \times SD/S$	(1)

$$LOQ = 10 \times SD/S$$

The computed LOD and LOQ values were 0.0015 ppm and 0.0049 ppm, respectively, but that LOD of S. A. Peterson (2007) was 0.0024 ppm [41].

4.1. Fish samples results

The present validated method was utilized to analyze 240 fish samples collected from various Yemeni fishing ports along the Arabian Sea and Red Sea coasts; the resulting data were depicted in Table 3, and visually represented as a histogram in Fig. 3. The data revealed that two samples out of 240 samples exceeded the YSMO current permissible limit for mercury in fish (0.5 ppm) [42]. The five samples were of the mercury (0.6906 ppm–1.3348 ppm) range.

As shown in Table 4, variation in Hg contamination also exists within the same family of fish. The computed T-test values, with a 95 % confidence limit (P < 0.05), revealed a significant difference in the total mercury concentration between Carangidae species from the Arabian Sea and the Red Sea. Nevertheless, a T-test was conducted to compare the mean Hg content in fish species of six other fish families in both the Arabian Sea and the Red Sea. However, the results did not reveal significant differences in the mean mercury content among the species. In general, the amount of total mercury in the 240 fish samples tested in our work ranged between (0.0212 ppm) and (1.3348 ppm).

This variation is expected due to various factors including, the type of fish, their age, size, and locations [10,11]. Previous reports revealed a variation of mercury amount in the Yemeni fish within the range of from 0.002 to 0.099 ppm [7,8,43].

5. Conclusion

240 Samples of fish Mercury concentrations were determined by using a Direct Mercury Analyzer (DMA-80). This method was fast, easy, simple, and rapid. The method showed high linearity. The results were reported as % RSD, reflecting high precession of the method. Recoveries of mercury from spiked real samples were highly accurate and appropriate methods were used. The levels of

mercuric concentration in the analyzed fish samples were found to be below the legal limits in YSMO. The fish samples in the Yemeni coast should be analyzed more often. This study improves the baseline data and information about the mercury concentration in the Yemeni fish.

Institutional review board statement

The study protocol was approved by the animal ethics committee of Biological Science, Sana'a University (ethical code: BAHSS102).

CRediT authorship contribution statement

Anass A. Alnedhary: Writing – review & editing, Resources, Methodology, Formal analysis, Data curation. Mahfoudh M. AL-Hammadi: Conceptualization. Abdualqawi A. Numan: Software, Resources, Methodology, Formal analysis. Fatima A. Murshed: Writing – review & editing, Software, Formal analysis. Ranya A. Alalie: Writing – original draft, Validation, Software.

Declaration of competing interest

The authors declare no conflict of interest.

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