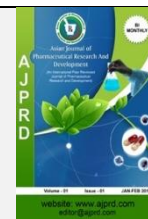


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Research Article

Analysis of Mercury in Fish, North Sumatera Indonesia by Atomic Absorption Spectrophotometer

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ABSTRACT

The maximum level of mercury permitted in fish by BPOM RI is 0.5 mg/kg. This study was conducted to determine the level of mercury in fish often consumed in Medan Indonesia. Fish samples were collected from 7 traditional fish market outlets and 1 supermarket in Medan in December 2019. Fish samples were dried using an oven at 103°C until a constant dry weight was attained. Then, the digestion process was carried out using concentrated nitric acid and perchloric acid. Determination of mercury level was carried out using CV-AAS method with 900 H Perkin Elmer atomic absorption spectrophotometer equipped with MHS-15 (Mercury Hydride System). The results showed that the level of mercury in pelagic fish was found significantly in yellowfin tuna (*Thunnus albacares*) of $27.3865 \pm 0.3326 \mu\text{g/kg}$; mackerel tuna (*Euthynnus affinis*) of $17.8570 \pm 0.0121 \mu\text{g/kg}$; skipjack tuna (*Katsuwonus pelamis*) of $17.4507 \pm 1.5893 \mu\text{g/kg}$; spanish mackerel (*Scomberomorus commersonii*) of $10.5767 \pm 0.1862 \mu\text{g/kg}$; grouper (*Epinephelus fuscoguttatus*) of $9.9736 \pm 0.9115 \mu\text{g/kg}$; mackerel (*Rastreliger kanagurta*) of $6.5364 \pm 0.1935 \mu\text{g/kg}$ and demersal fish were found significantly in red snapper (*Lutjanus campechanus*) of $14.0966 \pm 0.8555 \mu\text{g/kg}$; stingray (*Dasyatis sp*) of $61.3146 \pm 0.8149 \mu\text{g/kg}$; ariid catfish (*Arius thalassinus*) of $13.2533 \pm 0.7586 \mu\text{g/kg}$; black pomfret (*Parastromateus niger*) of $10.7755 \pm 0.4605 \mu\text{g/kg}$; sardine (*Sardinella sp*) of $6.5464 \pm 0.1036 \mu\text{g/kg}$. The results showed that the larger fish has the higher mercury level. The level of mercury in these analyzed fish samples was below maximum level permitted by BPOM RI.

Keyword: Mercury, Fish, Atomic Absorption Spectrophotometer-MHS 15.**ARTICLE INFO:** Received 17 April 2020; Review Completed 8 May 2020; Accepted 22 May 2020; Available online 15 June. 2020

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INTRODUCTION

Mercury is one of the most toxic metals found in nature and by the United States Government Agency places mercury as number three of the most toxic under arsenic and plumbum. Mercury poisoning is the result of exposure to mercury compounds or elements that produce various toxic effects depending on their chemical form and route of exposure. The main route of human exposure to methyl mercury is mainly through contaminated fish, seafood and wildlife. Methylmercury toxicity is associated with damage to the nervous system in adults, pregnant women and nervous system development disorders in infants and children¹.

Mercury is released into the environment through natural processes such as volcanoes, forest fires, ocean evaporation, geological activities and through anthropogenic activities such as coal mining, gold mining,

combustion of oil and natural gas, the chlor-alkali industry, dental amalgam and consumer product waste. Complex chemical transformations in the mercury cycle produce mercury in forms of inorganic (Hg^0 , Hg^+ , Hg^{2+}) and organic (CH_3Hg , $\text{CH}_3(2) \text{Hg}$)²⁻⁴.

In the mercury cycle in the aquatic environment, methylation of inorganic mercury (Hg^{2+}) into methyl mercury is considered one of the most important toxicologically transformations because not only bioavailability and methylmercury toxicity increase but in fact exposure to methylmercury in humans also increases. The mercury methylation process involves microorganisms that are able to oxidize and reduce mercury. Methylmercury is accumulated by fish and other marine mammals through the food chain which become a source of mercury exposure to human⁵.

The importance of fish consumption for health and nutrition has made fish as an important food source of protein, minerals, and fat which are important for human body. Fish contains functional compounds that are beneficial to health including long-chain Omega-3 unsaturated fatty acids consisting of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)⁶. In general, several studies agree that there are significant benefits from consuming long-chain fatty acids in fish for health improvement, but on the other hand that the negative effects of methylmercury can cause damage especially for vulnerable populations including pregnant women, nursing mothers and children. DHA, arachidonic acid (AA) and omega-6 PUFA are very important for the development of the central nervous system in human fetus. The neurotoxic effects of methylmercury in the fetus and developing babies are the most sensitive, therefore, pregnant and nursing women must be careful when consuming seafood^{7,8}.

Based on the data from BPS in 2018 (Statistics Indonesia) the average fish consumption in Indonesia is 324 grams of fish per person per week and data from BKPM in 2016 (Medan Food Security Agency) explains that the Medan people consume 91.69 grams of fish per person per day. The accumulation of mercury in the body of the fish is an important concern related to health. The threshold for the concentration of mercury in fish permitted by BPOM RI in 2017 (Indonesian Food and Drug Administration) is 0.5 mg/kg and WHO/FAO is 0.5-1.0 mg/kg.

In previous studies by Ade (2015)⁹; Rosmidah, (2004)¹⁰ determined mercury levels in fish obtained from Belawan Fish Auction Sites. Determination of mercury levels in several types of fish often consumed in Medan in traditional markets has never been done before. The purpose of this study was to determine the levels of mercury in several types of fish that are often consumed in Medan which obtained from traditional markets. Determination of mercury levels in fish performed by the CV-AAS method with 900H Perkin Elmer Atomic Absorption Spectrophotometer equipped with MHS-15 (Mercury Hydride System)¹¹.

MATERIALS AND METHODS

Materials and Instruments

The materials used in this study were analytical scales, ovens, whatman filter paper No.42, label paper and Perkin Elmer Pinaacle 900 H Atomic Absorption Spectrophotometer equipped with MHS-15 (Mercury Hydride System). Glassware and plastic containers soaked in 2% nitric acid and left overnight before rinsing it with deionized water. All reagents used were E. Merck (Germany) that have a high analytical level such as 65% nitric acid, 60% perchloric acid and 1000 mg/l Hg mercury nitric standard solution.

Sampling and sample preparation

The sampling method was conducted purposively based on the types of fish most often consumed by the people in Medan and the distribution of fish populations by region. Each fish represented one sample at random. Fresh fish samples were obtained from different traditional markets and supermarkets in Medan. Fish samples were packaged in plastic container, labeled and put in a freezer before

being taken to the laboratory. For sample preparation, the fish samples were left at room temperature then washed with distilled water, each fish is removed from its scales and the edible parts of the fish were cut into small pieces. Then the fish was blended. Then dried using an oven at 103°C. Samples preparation and analysis of mercury in several types of fish was carried out in the ProLing Laboratory, Department of Fisheries Resources Management, Faculty of Fisheries, IPB University.

Determination of Mercury in Fish

The method used in this study based on the procedure as described by American Public Health Association, (2017)¹². One gram of sample transferred into a 100 ml Erlenmeyer flask. Added 5 ml HNO₃ and boiled on a hot plate at 80°C until the remaining solution only 1-2 ml. Added 10 ml of HNO₃ and 10 ml HClO₄ to the cooled solution. Then reheated on a hot plate at 80°C until HClO₄ vapor disappeared. Then added 50 ml of deionized water to the cooled solution and boiled to remove the chlorine compound and nitrogen oxides. The solution was filtered while rinsing with deionized water until 100 ml volume of filtrate obtained. Then the solution was measured by Perkin Elmer Pinaacle 900 H Atomic Absorption Spectrophotometer equipped with MHS-15 (Mercury Hydride System) at wavelength of 253.65 nm.

Method validation

Method validation was performed with several parameters such as linearity, accuracy, precision, detection limit and quantization limit. Linearity was obtained by preparing a calibration curve of several standard solution concentrations and calculating the correlation coefficient (r). The accuracy was determined by adding a standard solution to the sample and then proceeded with the sample analysis procedure as previously done using a cold vapor atomic absorption spectrophotometer, while precision is measured as a relative standard deviation. Determination of detection limit and quantitation limit based on the calculation of the calibration curve obtained.

Calibration Curve

Ten milliliters of primary standard solution of 1000 mg/l mercury nitrate was pipetted into a 100 ml flask (100 mg/l concentration). Then 0.5 ml of 100 mg/l solution was pipetted into a 100 ml flask (0.5 mg/l concentration). From 0.5 mg/l solution, 1.0 ml; 2.0 ml; 4.0 ml; 10.0 ml of solution was pipetted and put into a 100 ml flask so that the concentration of the solution obtained respectively 5 µg/l, 10 µg/l, 20 µg/l, 50 µg/l and these solution is mercury standard solution used.

The calibration curve is obtained by entering a certain amount of the standard solution to the system and proceed with intensity measurement of ions number which is calculated by the detector at every second or Counts per Second (CPS)¹³. From calibration curve measurement obtained mercury standard solution regression equation $Y = 0.01241X + 0.00998$. The concentration of mercury in sample (X) can be obtained from the calculation of regression equation if the Y value (absorbance) is known. The increase in mercury concentration in the sample (X) is proportional to intensity increasement (Y).

Mercury Level in Fish

$$\text{Mercury level } (\mu\text{g/mL}) = \frac{X(\mu\text{g/mL}) \times V(\text{mL}) \times F_p}{W(\text{g})}$$

X = Concentration of analyte in sample solution

V = Total volume of sample solution examined

F_p = Dilution factor of the decomposition result

W = Sample weight

RESULT AND DISCUSSION

From the validation results, it was showed that trueness (% recovery) and precision obtained were excellent. The detection limit and quantification limit achieved were low enough and suitable for determining mercury levels in fish at the low levels found in the sample. The validation methods performed are shown in Table 1.

Table: 1 Validation performance

Parameters	Mercury
Working range ($\mu\text{g L}^{-1}$)	0.0 – 50.0
Correlation coefficient, r	0.999478
Recovery (%)	103.1928
RSD (%)	1.7615
LOD ($\mu\text{g kg}^{-1}$)	1.6921
LOQ ($\mu\text{g kg}^{-1}$)	5.0406

The result showed from 11 analyzed fish samples had different levels of mercury which can be seen in Table 2 and Figure 1.

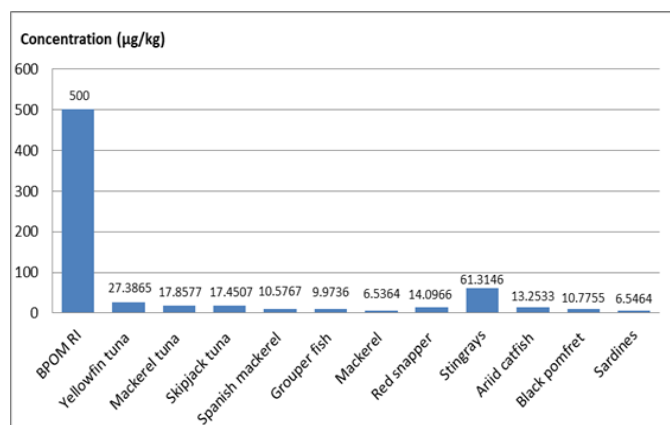


Figure: 1 Mercury levels in Fish

Table: 2. Mercury Levels in Fish Samples Marketed in Medan

No	Fish Samples	Market of Sample Collection	Length (cm)	Weight (kg)	Mercury Level ($\mu\text{g/kg}$)
Pelagic Fish					
1	Yellowfin tuna (<i>Thunnus albacares</i>)	Berastagi Supermarket	53	1.5	27.3865 ± 0.3326
2	Mackerel tuna (<i>Euthynnus affinis</i>)	Sei Sekambing traditional market	50	1.38	17.8570 ± 0.0121
3	Skipjack tuna (<i>Katsuwonus pelamis</i>)	Petisah traditional market	44	1.25	17.4507 ± 1.5893
4	Spanish mackerel (<i>Scomberomorus commersonii</i>)	Petisah traditional market	76	2.2	10.5767 ± 0.1862
5	Grouper (<i>Epinephelus fuscoguttatus</i>)	Sambu traditional market	44	0.82	9.9736 ± 0.9115
6	Mackerel (<i>Rastreliger kanagurta</i>)	Padang Bulan traditional market	23	0.15	6.5364 ± 0.1935
Demersal Fish					
7	Red snapper (<i>Lutjanus campechanus</i>)	Glugur traditional market	80	8.2	14.0966 ± 0.8555
8	Stingray (<i>Dasyatis sp</i>)	Glugur traditional market	64	5.9	61.3146 ± 0.8149
9	Ariid catfish (<i>Arius Thalassinus</i>)	Selayang traditional market	37	0.77	13.2533 ± 0.7586
10	Black pomfret (<i>Parastromateus niger</i>)	Johor traditional market	34	0.84	10.7755 ± 0.4605
11	Sardines (<i>Sardinella sp</i>)	Selayang traditional market	17	0.09	6.5464 ± 0.1036

Note: Data is the means of three replicates (n=3)

The levels of mercury in fish samples ranged from 6.5364 – 61.3146 $\mu\text{g/kg}$. The highest level of mercury 61.3146 $\mu\text{g/kg}$ was found in stingray which obtained from Glugur Traditional Market while the lowest mercury level was

6.5364 $\mu\text{g/kg}$ in mackerel obtained from Padang Bulan Traditional Market. High level of mercury in stingray indicated that accumulation of mercury was absorbed more compared to other fish. This data also showed that fish

obtained in traditional markets were exposed to mercury contamination. The amount of mercury level in fish samples were still relatively small when compared to the maximum limit of mercury contamination in processed food as stipulated by the Head of BPOM RI Regulation No.23 of 2017 at 0.5 mg/kg (500 µg/kg).

From the data Table 2 shows that fish such as Yellowfin tuna, Mackerel tuna, Skipjack tuna, Spanish mackerel, Red snapper, Stingray have a larger size and weight than other fish and show higher levels of mercury. The difference in mercury levels is due to the correlation of mercury concentrations with the age/size of the fish. Fish of different sizes show a correlation to mercury concentrations. It is found that mercury levels appear to increase with the size of fish where larger fish have higher mercury concentrations than smaller fish. The different accumulation and biomagnifications processes in fish will correlate mercury concentrations with fish size^{14, 15}.

Data also shows that the highest levels of mercury are found in stingray (61.3146 µg/kg). Stingray is not predatory pelagic fish but demersal species that live on the seabed. The high levels of mercury in stingray is not only due to size and weight but can be caused by eating habit factor. In general, fish eating habits can be divided into three, namely (1) benthivores and planktivores, (2) carnivores, and (3) omnivores¹⁶. Stingray is classified as benthivorous and planktivorous fish. Eating habit of stingray is in accordance with research by Ashraf.et.al, (2012)¹⁷ which states that fish with benthivorous and planktivorous eating habit accumulate pollutant metals higher than carnivorous and omnivore fish. The accumulation of pollutant metals in marine biota is influenced by internal and external factors. Internal factors are length, weight, age, and speed of metabolism. External factor is eating habits¹⁶.

Previous study conducted by Nurul.et.al, (2014)¹⁸ in stingrays obtained levels of mercury around 384- 548 (µg/kg). The level of mercury from that study was quite high compared to the level of mercury in this study which was 61.3146 (µg/kg). Differences of mercury level in stingray species in different places indicate different amounts of mercury in the aquatic environment where the fish habitat is. The first factor that causes differences in mercury levels in fish is the source of emissions from anthropogenic activities in each country is different. According to UNEP, (2013)³ the largest proportion of anthropogenic mercury emissions to the atmosphere comes from Asia, which accounts for around 50% of the global total. The majority of Asian emissions come from East and Southeast Asia. China accounts for three-quarters of East and Southeast Asia emissions, or about one third of the global total. Second, chemical and biological controls in the aquatic environment such as dissolved organic matter (DOM), sulfate reduction, and sulfide inhibition, alkalinity, calcium, chlorophyll *a*, conductance, magnesium, pH, total nitrogen, and total phosphorus correlate well in the formation of methylmercury by microorganisms⁴. Third, Fish of different sizes show a correlation to mercury concentrations. It is found that mercury levels appear to increase with the size of fish where larger fish have higher mercury concentrations than smaller fish. The different accumulation and biomagnification processes in fish will correlate mercury concentrations with fish size^{14, 15}.

CONCLUSION

From the research, it was obtained different levels of mercury present in fish marketed in Medan ranging from the highest in stingray (61.3146 µg/kg) to the lowest level found in Mackerel (6.5364 µg/kg). This level is still safe because the maximum permitted level of mercury in fish regulated in BPOM RI is 0.5 mg/kg.

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REFERENCES

1. Kevin MR, Ernest MW, Miaocong W, Chris G, Eric RB. Environmental Mercury and Its Toxic effects. Review. Journal Preventive Medicine and Public Health. 2014; 47:74-83.
2. Clarkson TW, Magos L. The Toxicology of Mercury and Its Chemical Compounds. Critical Reviews in Toxicology. 2006; 36:609–662.
3. United Nations Environment Programme. The Global Atmosphere Mercury Assessment. Sources, Emissions and Transport. Division of Technology, Industry and Economics (DTIE). Chemicals Branch. Geneva. Switzerland. 2013.
4. Zillioux EJ. Mercury in Fish: History, Sources, Pathways, Effects, and Indicator Usage. Journal Environmental Indicators. 2015; 3(2):743-768.
5. Zurahanim FA. Exposure assessment for mercury and other metals in commonly consumed fish of west Peninsular Malaysia. PhD in Applied Science. Faculty of Education, Science, Technology & Mathematics University of Canberra. 2014.
6. Myers GJ, Davidson PW, Strain JJ. Nutrient and methylmercury exposure from consuming fish. The Journal of Nutrition. 2007; 137(12): 2805-2815.
7. Chan HM. Mercury in Fish: Human Health risks. Encyclopedia of Environmental Health. 2011; 697-704.
8. Cladis DP. Fatty Acids and Mercury In Seventy Seven Species of Commercially Available FinFish In The United State. Master of Science Purdue University West Lafayette, Indiana. 2014.
9. Ade IN. Efektivitas Larutan Jeruk Nipis terhadap Penurunan Kadar Merkuri (Hg) pada ikan Tongkol (*Euthynnus*). Skripsi. Fakultas Kesehatan Masyarakat. Universitas Sumatera Utara. 2015.
10. Rosmidah S. Analisa Kandungan Merkuri pada ikan (*Pisces*) dan Kerang (*Mollusca*) di TPI (Tempat Pelelangan Ikan) Belawan. Skripsi. Fakultas Kesehatan Masyarakat. Universitas Sumatera Utara. 2005.
11. World Health Organization. Elemental mercury and Inorganic Mercury Compounds: Human Health Aspects. Concise International Chemical Assessment Document 50. 2003.
12. American Public Health Association. Standard Method for the examination water and wastewater. 23rd Edition. 2017; 3114-B, 3030-H.
13. Rohman A. Validasi dan Penjaminan Mutu Metode Analisis Kimia. Yogyakarta. UGM press. 2016; 87-109.
14. Lange TR, Royal HE, Connor LL. Influence of Water Chemistry on Mercury Concentration in Largemouth Bass from Florida Lakes. Journal Transaction of the American fisheries Society. 1993; 122(1):74-84.
15. Storelli MM, Giacomini-Stuffler R, Marcotrigiano GO. Relationship between Total Mercury Concentration and Fish Size in Two Pelagic Fish Species: Implications for Consumer Health. Journal of Food Protection. 2006; 69(6):1402–1405.
16. Suratno, Cordova MR, Arinda S. Kandungan Merkuri dalam Ikan Konsumsi di Wilayah Bantul dan Yogyakarta. Oseanologi dan Limnologi Indonesia. 2017; 2(1):15–23.

17. Ashraf MA, Maah MJ, Yusoff I. Bioaccumulation of Heavy Metals in Fish Species Collected From Former Tin Mining Catchment. *Int. J. Environ. Res.* 2012; 6(1):209-218.
18. Nurul IA, Mohd FMN, Wan RWM, Hamdan J, Ismail I, Wan NFWA, Yuvaneswary V, Mohd HH. Mercury levels of marine fish commonly consumed in Peninsular Malaysia. *Environmental Science and Pollutan International.* 2015; 22(5):3672-86.

