# A modified method for the determination of organic mercury in biota [great cormorants (phalacrocorax carbo)] by advanced mercury analyzer

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**ABSTRACT**

**Background:** Contagion of aquatic ecosystems to heavy metals especially mercury (Hg) has risen concerns about healthiness of marine organisms. Organic mercury compounds are highly toxic for animals, and its detection in various samples is frequently needed. In present study we have described a new method for measurement of organic mercury and total mercury concentration in great cormorants (phalacrocorax carbo) of southern coasts of Caspian Sea. Also, resultant values have been compared with world health standards.

**Methods:** 18 great cormorants were hunted randomly in southern coasts of Caspian Sea during March 2009. Analysis of organic and total mercury was performed on samples from their liver, kidney and muscle, with Advanced Mercury Analyzer (Model Leco, AMA 254) for the first time in Iran. This method can be used for others biota.

**Results:** Mean concentrations of total mercury were 5.67, 3.59 and 2.26 mg/kg in animal liver, kidney and muscle respectively; from which 82, 79 and 58 percent were comprised from organic mercury respectively. Comparison of resultant figures showed statistically significant differences ($P<0.05$); but no differences were found between different sexes ($P=0.69$).

**Conclusion:**Total mercury concentrations in tissues of great cormorant were outstandingly higher than WHO, FAO and EPA standards. It is a serious threat for end users of the bird meat especially insubstantial humans.

**Key words:** Great cormorant, total mercury, Organic mercury, AMA 254, Caspian Sea.

## INTRODUCTION

Mercury is a heavy metal found in nature in organic and mineral forms with unique nature (1,2). Its bioaccumulation, global distribution and health hazards have received vast attentions (3). Organic mercury compounds are more toxic than mineral ones. Di methyl mercury [Hg $(CH_3)_2$] is the most noxious one among organic mercury compounds. Mercury compounds can initiate methylation in nature particularly in aquatic ecosystems. Indeed the most important causes of methylation are bacteria and yeasts living in water and its precipitants. Methyl mercury is methylated by bacteria and is converted to Di methyl mercury which is the most toxic one among mercury compounds. Methylation reaction is hastened in acidic water and waters with low oxygen content owing to heavy

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concentration of microorganisms (4). Methyl mercury has high affinity to sulphydryl groups of proteins; so it relocates in food chain and accumulates in animal tissues (5).

Mercury poisoning has been observed in many countries chiefly in sea based products. Neurologic, cardiovascular and renal abnormalities have been seen in mercury poisoning (6 and 7). Also there are some reports about its carcinogenicity and mutagenesis in experimental animals. Absorption of organic mercury compounds take place from GI mainly. It is generally excreted from urine. Mineral mercury compounds are eliminated from body faster; but organic compounds may stay more in body (4).

Organic mercury compounds may accumulate in body and have unfavorable upshots on aquatic ecosystems; so measurement of mercury in various tissues of animals is needed for monitoring of its status on ecosystems. Various tissues of marine birds are suitable biomarkers for evaluation of mercury concentration in aquatic ecosystems (8).

Aquatic ecosystems of Iran may be contaminated to mercury similarly. Birds are appropriate for ecotoxicology because of wide geographic distribution, relatively prolonged life span and their position in food chain (10); so we used great cormorant (phalacrocorax carbo) for determination of mercury levels in aquatic ecosystems of Iran as it had been proposed by others (11 and 12).

## MATERIALS AND METHODS

Current study was performed in beach of Caspian Sea at Anzali and Gomishan's pools. These ponds have international values and are host of numerous migrant birds at winters.

After taking required credential from government administrative center 18 Great Cormorants included 7 males and 11 females were caught randomly from various places of studied pools. They were transferred to laboratory rapidly where they were dissected and samples from their kidney, liver and muscles were taken. The samples were kept in -20°C until analysis.

***Analysis of total mercury***

Accumulated mercury level was measured using a LECO AMA 254 Advanced Mercury Analyzer (U.S.A). Accuracy of total Hg analysis was checked by running three samples of Standard Reference Materials (SRM), National Institute of Standard and Technology (NIST), SRM 1633b, SRM 2709 and SRM 2711 in seven replicates. Recovery varied between 96.4% and 103.6%. 2 grams from each sample was placed in an aluminum foil and weighted. The samples were incubated in freeze-drier appliance for 48 hours to be dried. Then they were weighted again and their humidity was determined. After complete milling of the samples 0.03-0.05 grams of dried tissue were used for total mercury determination with Advanced Mercury Analyzer (AMA 254). The apparatus can detect mercury in 5µg/kg to 5 mg/kg. The results were reported as mg/kg wet weight.

***Analysis of organic mercury***

Modified method of EPA 7473 is used for determination organic mercury (guidance: to receive more details and calibrate AMA 254, please search "method EPA 7374" in Google scholar. So you can receive latest edited file in PDF format). In our method that is preformed for the first time in Iran and is modification of previous methods 0.5 grams of each sample (wet tissue) was mixed with 10 ml HCl, 0.5 grams Sodium bromide and 10 ml toluene in a capped glass tube. The tubes were trembled with a shaker for 20 minutes to digest the tissues; then centrifuged for 20 minutes at rate 4500 rpm. Upper organic phase contained toluene was removed. Organic mercury in tissues was transported to toluene layer; but the layer cannot be injected into instrument because of high volatility and potential of damage to internal surface of the instrument. So organic phase were mixed with cystein solution 1% w/v twice in order to organic mercury be transferred from organic phase to aqueous phase. Each stage was accompanied by shaking for 10 minutes, centrifuging for 10 minutes, and removing lower aqueous phase. Finally 200 µl of aqueous phase was injected into Advanced Mercury Analyzer (AMA 254) immediately. Accuracy and precision of the above mentioned method was tested against with standard sample of methyl mercury chloride. Recovery varied between 98.2% and 101.8%.

***Statistical analysis***

Statistical analysis of resulting data was performed by SPSS software version 17. Normality of data was checked by Shapiro-Wilk test. Non-parametric tests were used for comparison of mercury level in various tissues. Confidence interval was determined as 95%.

## RESULTS

Total and organic mercury level in various tissues of Great Cormorant was determined with Advanced Mercury Analyzer (AMA 254) in current study. The resulting data was shown in table 1. As one can see organic mercury comprise 82, 79, and 58 percent of total mercury level in liver, kidney, and muscle of the bird respectively. There is statistically significant difference between total and organic levels of mercury in assorted tissues with confidence level of 95%. The levels were higher in liver match up to other tissues (chart 1) ($P<0.05$). There was no statistically significant difference in mercury levels in different genders ($P=0.69$). At last, mercury level in various tissues of Great Cormorant more than ever liver was exceedingly higher than levels recommended by EPA, FAO, and WHO.

**Table 1:** mean total and organic mercury levels in tissues of Great Cormorant (mg/kg w.w)

| Tissue | Total-Hg (Mean ± SEM) | Organic-Hg (Mean SEM) | Hg Max-Min (Range) | Organic-Hg Max-Min (Range) |
|---|---|---|---|---|
| Liver (n=18) | 5.67 ± 0.19 | 4.64 ± 0.33 | 0.90 -12.71 (11.81) | 0.74 – 9.87 (9.13) |
| Kidney (n=18) | 3.59 ± 0.65 | 2.85 ± 0.72 | 4.46 – 7.64 (3.18) | 3.52 – 6.04 (2.52) |
| Muscle (n=18) | 2.26 ± 0.38 | 1.32 ± 0.28 | 0.86 – 11.8 (10.22) | 0.50 – 6.43 (5.93) |

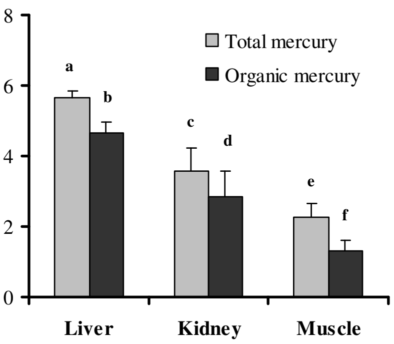


***Fig1:** total and organic mercury levels in tissues of Great Cormorant (mg/kg w.w)*

## DISCUSSION

Mercury is a highly neurotoxic element which may accumulate in animal's tissues particularly liver and kidney. Methyl chloride has high affinity to sulphydryl groups; hence it can convey in food chain and mount up in tissues (3). Analyzing and monitoring of heavy metals including mercury in various tissues of assorted animals now have crucial importance in view of ecosystem management as well as human food health (18). Numerous previous studies have showed that liver receive more mercury in comparison with other tissues such as muscle. Present study showed that total and organic mercury levels in liver and kidney are higher than muscle too.

In some studies organic mercury level in liver had been lower than that of muscle; a finding which may be as a result of regular demethylation in the liver that convert organic mercury (methyl chloride) to inorganic less toxic forms (19,20). Kannan and his coworkers have suggested that ratio of methyl mercury to total mercury may be as high as 0.8 owing to presence of cystein in muscle tissue. Methylation is performed in muscle too and it had been expected that muscle tissue had more organic mercury (21); however current study found higher ratio of methyl mercury to total mercury in all examined tissues. It may attributable to less mobility of the birds in winters, nutrition from fish, presence of elevated organic mercury in environment due to human manipulations in ecosystems, and transporting the mercury to liver by the bird to lower its toxicity. By increasing age of pools more anaerobic microorganisms live in precipitates of pools; change inorganic mercury to organic one. The fact is particularly true about Anzali pool (22). Another reason for low organic mercury in bird's muscle may be presence of high oxygen in muscles owing to its less motility in winters. Oxygen can trigger oxidation of organic mercury; mostly in muscle tissue (23). Recent research in United State have demonstrated high mercury level in liver of sea birds in south Florida bring about neurologic and reproductive sequelae, and possibly has diminished the bird population (24). Organic mercury usually accumulated in liver and kidney of birds; since liver is a main organ for detoxification, metabolism, and excretion of methyl chloride in bile. Moreover mercury salts is excreted from liver and kidney via bile and feces (25).

Some part of high mercury level in organs of Great Cormorant in March may be attributable to their higher weight and age during last months of migration which make less motility and consequently less mercury excretion inevitable. In this period, refurbish of feathers a usual way for excretion of heavy metals in birds is stumpy too.

Permissible level of mercury in body of individuals is 500 and 300 ng/g according to FAO and WHO, and EPA respectively (26,27). Our found level of mercury in the bird liver is 11-fold match up to FAO and WHO standards. The ratios for kidney and muscle are 7 and 4.5 fold respectively. If EPA standards are considered, the ratios for liver, kidney and muscle are 20, 11, and 7 fold. Results of present study rise attentions about a serious hazard especially for consumers of the bird meat. Governmental authorities should take measures to manage ecological contagion of Caspian Sea, an international ecosystem for bird's migration.

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## REFERENCES

1. Storelli MM, Giacominelli-Stuffler R, Marcotrigiano GO. Total and methyl mercury residues in cartilaginous fish from Mediterranean Sea. Marine Pollution Bulletin. 2002; 44(12): 1354-58.
2. Zalups RK. Molecular interactions with mercury in the kidney. Pharmacol Rev. 2000; 52(1): 113-43.
3. Mazloomi S, Esmaili-Sari A, Ghasempouri M, Omidi A. Mercury distribution in Liver, Kidney, Muscle and Feathers Caspian Sea Common Cormorant (phalacrocorax carbo). Research Journal of Environmental Sciences. 2008; 2(6): 433-437.
4. Esmaiili-Sari A, Noori-Sari H, Esmaiili-Sari A. Mercury in the environment,Rasht: 2007.p.1-10, (in persian).
5. Ochoa-acuna H, Sepulveda S, Gross T. Mercury in feathers from Chilean birds: influence of location, feeding strategy, and taxonomic affiliation. Marine Pollution Bulletin, 2002; 44: 340-349.
6. Vupputuri S, Longnecker MP, Daniels JL, Xuguang G, Sandler DP. Blood mercury level and blood pressure among US women: results from the national Health and Nutrition Examination Survey 1999-2000. Environmental Research. 2005; 97(2): 195-200.
7. Canli M, Atli G. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environmental Pollution. 2003; 121(1): 129-36.
8. Veerle J, Dauwe T, Rianne P, Lieven B, Ronny B, Marcel E. The Important of Exogenous contamination on Heavy Metal Levels in Bird Feathers. A Field Experiment With Free-living great tits, Parus majer. Environmental Monitoring and Assessment. 2004; 6: 356-360.
9. Yazdandad H. A study on biological and ecological attributes of coot (Fulica atra) in wetlands of northern Iran. Journaln of Agricultural science and natural resource. 2007; 14: 134–144 (in persian).
10. Spalding M, Bjork R, Powell G, Sundlof S. Mercury and cause of death in great white

herons(Ardea herodias occidentalis). Wildlife management. 1994; 58: 735-739.
11. Saeki K, Okabe E, Kim S, Tanabe M, Fukuda R, Tatsukawa B. Mercury and cadmium in common cormorants (Phalacrocorax carbo). Environmental Pollution. 2000; 108: 249-255.
12. Mansoori J. A Guide to the Birds of IRAN.Tehran; Farzaneh Books, 2008. p. 46-47, ( in persian).
13. Mansoori J. The Avian Community of Five Iranian Wetlands, Miankaleh, Fereidoonkenar, Bujagh, Anzali and Lavandevil, in the South Caspian Lowlands. Podoces. 2009; 4(1): 44-59.
14. Maggi C, Teresa-Berducci M, Bianchi J, Giani M, Campanella L. Methylmercury determination in marine sediment and organisms by Direct Mercury Analyser. Analytica Chimica Acta. 2009; 641: 32-36.
15. Marrugo-Negrete J, Olivero-Verbel J, Lans-Ceballos E, Norberto-Benitez L. Total mercury and methylmercury concentrations in fish from the Mojana region of Colombia. Environmental Geochemistry and Healt. 2007; 30: 21-30.
16. Akagi H, Castillo S, Cortes-Maramba N, Francisco-Rivera A, Timbang D. Health assessment for mercury exposure among school children residing near a gold processing and refining plant in Apokon, Tagum, Davao del Norte, Philippines. Science of the Total Environment. 2000; 259: 31-43.
17. Sakamoto M, Kaneoka T, Murata K, Nakai K, Satoh H, Akagi H. Correlations between mercury concentrations in umbilical cord tissue and other biomarkers of fetal exposure to methylmercury in the Japanese population.¬¬ Environmental Research. 2007; 103: 106-111.
18. Romeo M, Siau Y, Sidoumou Z, Gnassia-Barelli M. Heavy metal distribution in different fish species from the Mauritania coast. Science of the Total Environment. 1999; 232(3): 169-175. species collected in French Guiana (Amazonian basin). Science of the Total Environment. 2006; 368(1): 262-70.
19. Regine MB, Gilles D, Yannick D, Alain B. Mercury distribution in fish organs and food regimes: Significant relationships fromtwelve
20. Bebianno M.J, Santos C, Canario J, Gouveia N, Sena-Carvalho D, Vale C. Hg and metallothionein-like proteins in the black scabbard fish Aphanopus carbo.Food and Chemical Toxicology. 2007; 45(8): 1443-52.
21. Kannan K, Smith RG, Lee RF, Windom HL,Heitmuller PT, Macauley JM, et al. Distribution of total mercury and methyl mercury in water, sediment, and fish from south Florida estuaries.Arch Environ Contam Toxicol. 1998; 34(2):109-18.
22. Farkas A, Salanki J, Specziar A. Age and size-specific patterns of heavy metals in the organs of freshwater fish Abramis brama L. populating a low-contaminated site. Water Research. 2003; 37(5): 959-64.
23. Freadman MA. Role partitioning of swimming musculature of stripedBassMorone saxatilisWalbaum and Bluefish, Pomatomus saltatrix L. Journal of Fish Biology. 2006; 15(4): 417-23.
24. Sundlof F, Spalding M, Wentworth J, Steible C. Mercury in livers of wading birds(Ciconiiformes) in southern Florida. Bulletin of Environmental Contamination and Toxicology. 1994; 27: 299-305.
25. Boening W. Ecological effects, transport, and fate of mercury: A general review Chemosphere. 2000; 40: 1335-1351.
26. Ebinghaus R, Hintelmann H, Wilken RD. Mercury cycling in surface waters and in the using an automatic mercury analyzer. Food Chemistry. 2007; 100(2): 853-58.
27. Jewett SC, Duffy LK. Mercury in fishes of Alaska, with emphasis on subsistence species. Science of the Total Environment. 2007; 387(1-3): 3-27.