MERCURY AND METHYLMERCURY ANALYSIS IN TWO ISLANDS OF THE CARIBBEAN, COLOMBIA: RISK ASSESSMENT INDEXES

Data de submissão: *Data de aceite: 03/07/2023*

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ABSTRACT: Marine ecosystems are relevant in the biogeochemical cycle of mercury as they house its chemical speciation, a natural and necessary process altered by the current rate of contamination that compromises environmental health and food safety and consequently the food security of populations. This document presents an analysis of the health risk from the consumption of fish contaminated with xenobiotics in two islands of the Caribbean Sea. Consumption preferences were recorded through a survey identifying the five most consumed fish species, after capture, muscle samples were taken to evaluate the concentration of mercury and methylmercury. Exposure levels and health risk indices associated with consumption were calculated. The results indicate that *Haemulon flavolineatum* presented the highest concentration, between 1.66 to 1.82 Hg mg Kg^{-1}, in four of the evaluated species methyl-mercury presented high values between 0.15 to 1.34 CH_3-Hg mg Kg^{-1}. A difference was identified with respect to the pattern of incremental concentration by trophic level reported in the literature, possibly related to feeding habits and high concentrations of mercury in sediments (the latter was recorded in a parallel study).

With the exception of *Sphyraena barracuda*, a carcinogenic health risk was observed in the evaluated species that exceeds the limit proposed by the EPA.
KEYWORDS: Environmental health; Caribbean sea; Fishery resources; Food security.

ANÁLISIS DE MERCURIO Y METILMERCURIO EN DOS ISLAS DEL CARIBE, COLOMBIA: ÍNDICES EVALUACIÓN DEL RIESGO.

RESUMEN: Los ecosistemas marinos son relevantes en el ciclo biogeoquímico del mercurio ya que alojan su especiación química, proceso natural y necesario alterado por la tasa actual de contaminación que compromete salud ambiental e inocuidad de alimentos y consecuentemente la seguridad alimentaria de las poblaciones. Este documento presenta un análisis del riesgo para la salud por consumo de pescado contaminado con xenobióticos en dos islas del Mar Caribe. Las preferencias de consumo se registraron mediante encuesta identificando las cinco especies de pescado más consumidas, luego de capturadas se tomaron muestras de músculo para evaluar la concentración de mercurio y metilmercurio. Se calcularon los niveles de exposición y los índices de riesgo para la salud asociados al consumo. Los resultados indican que *Haemulon flavolineatum* presentó la mayor concentración, entre 1.66 a 1.82 Hg mg Kg^{-1}, en cuatro de las especies evaluadas el metil-mercurio presentó valores elevados entre 0.15 a 1.34 CH_3-Hg mg Kg^{-1}. Se identificó una diferencia con respecto al patrón de concentración incremental por nivel trófico reportado en la literatura, posiblemente relacionado con los hábitos de alimentación y las concentraciones elevadas de mercurio en sedimento (registradas en un estudio paralelo). A excepción de *Sphyraena barracuda*, se observó un riesgo cancerígeno para la salud en las especies evaluadas que supera el límite propuesto por la EPA.
PALABRAS CLAVE: Salud ambiental; Mar del Caribe; Recursos pesqueros; Seguridad alimentaria.

1 I INTRODUCTION

Heavy metal contamination of ecosystems that provide the food production ecosystem service is an issue of concern because compromises food security, and environmental and public health locally and globally [1,2]. For aquatic ecosystems, the allochthonous occurring mercury concentrations usually represent the lowest contribution compared to other chemical configurations of mercury, however environmental factors trigger the *in situ* methylation and induce the mercury transformation to its higher toxic form, methyl-mercury, and compels mercury circulation through the trophic net leading it as far as the tissues of fish captured for food [3, 4].

The ocean plays a central role in the biogeochemical cycle of mercury through many biotic and abiotic processes (volcanic activity, oxidation or reduction, biotransformation, sedimentation, and resuspension) that results in its chemical speciation, however currently, the anthropogenic activity influences two patterns of this cycle: the temporal and the spatial, even at the global scale affecting the transit between the source and the sink of mercury and exposing the aquatic biota, and the human being itself, to higher xenobiotic concentrations [5-9]. The presence of mercury and methyl-mercury around the Caribbean islands places the local population and the marine ecosystem at imminent risk by direct and indirect exposition as biomagnification or bioaccumulation of the xenobiotic occurs through the food web, and finally, results in the human exposition through water catchment or fish consumption [10-13].

Different factors such as the scarcity of local reliable data, the variability of environmental contexts, and the government priorities have been particularities difficulting the investigation of the cycling of mercury in the tropics, while the increment of environmental risk continues, there is important to assess the extent of biogeochemical mercury cycling alteration that conduces to elevate mercury concentrations in coastal environments, and also its biodisponibility potential [12, 14].

Providence island belongs to the Colombian archipielago named San Andrés, Providence, and Santa Catalina, it is conformed of three principal islands inhabited by the raizal community and migrants from the continental territory; the archipielago has 180,000 km^2 of submarine areas part of the UNESCO World Network of Biosphere Reserves (WNBR) since 2000 named Seaflower Biosphere Reserve, with high diversity, ecosystemic richness and importance in connectivity acting as a corridor for many marine species, the increase of anthropic activity in the area affects the environment and the population that consumes artisanal fishery products [15-17].

In the context of the 2019 version of the Seaflower Expeditions were taken samples for valuation of insular territory environmental quality of Providencia and Santa Catalina islands, the project pretends to supply inputs for environmental management strategies tuned with the environmental context around the Colombian islands [18]. In this work, a preliminary approximation to health risk estimation was carried out for the population consuming fish potentially contaminated with mercury and methyl-mercury in the islands, there have been few studies focused on risk assessment for the population in the Colombian Caribbean islands exposed to heavy metals whose main economic activities are fishing and tourism.

2 I METHODOLOGY

2.1 Study area

Providencia and Santa Catalina islands are in the Caribbean Sea lying along the Lower Nicaraguan Rise [19]. The archipelago has an extension of about 17 Km^2 of land over the sea and a mean altitude of 2 m.a.s.l., and approximately 61,280 inhabitants according to the National Administrative Department of Statistics (DANE for Spanish initials) [20]. The UNESCO Seaflower Biosphere Reserve and the Old Providence McBean Lagoon National

Natural Park are located on the northeast side of Providencia and Santa Catalina islands and are centered on this group of islands (Figure 1).

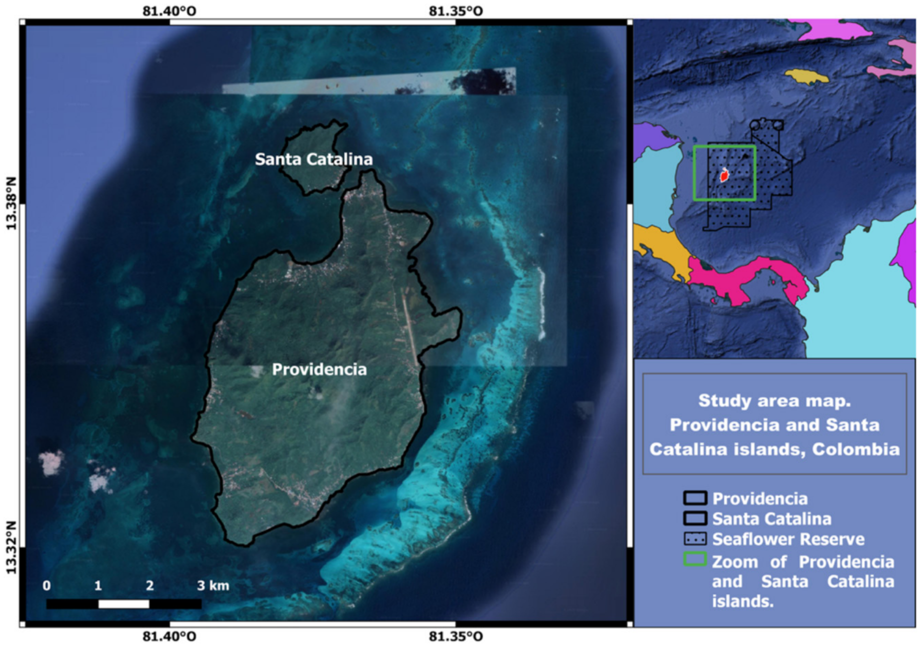

Figure 1. Location of Providencia, Santa Catalina and the Seaflower Biosphere Reserve in South America.

2.2 Consumption surveys

To determine the consumption preferences a survey was randomly applied over the population of Providencia and Santa Catalina islands, to the people between 14 and 80 years who were permanent inhabitants, 4,463 persons. The sample size (*n*=50) was calculated with Equation 1 [21]:

$$n = \frac{N * Z_a^2 * p * q}{d^2 * (N-1) + Z_a^2 * p * q} \quad (1)$$

where *n:* sample size; *N:* population size; Z_a: confidence level; *p:* success probability; *q:* failure probability; *d:* maximum permissible error.

Table 1 presents the values of the parameter and the result of the calculation of sample size.

n	*N*	Z_a	*p*	*q*	*d*
50.1	4463	1.96	0.95	0.05	0.06

Table 1. Parameters for sample size calculation.

2.3 Sampling

A sample of the fish caught by artisanal fishermen was taken in September 2019 based on the survey about consumer preferences for selecting the fish species. A 200 g sample of muscle tissue was taken from the 5 fish species reported with the highest consumption frequency. The samples were transported under handling and cold chain (-4°C) to a certificated laboratory for mercury quantification in triplicate for each species (n=3 specimens by species), the mercury determination was done by atomic absorption spectrometry with a detection limit of 0.02 Hg mg Kg^{-1} w.wt. [22]. Methyl-mercury concentrations were determined by gas chromatography with electron-capture detection and a low-limit detection of 0.086 CH_3-Hg mg Kg^{-1}. Recoveries accepted to validate determinations were between 96.2% and 102.1% for Hg, and 90.3% and 101% for CH_3-Hg. All samples were run in batches that included blanks, and a standard calibration curve made of solution traceable to NIST Certified Reference Materials for mercury and duplicates. The statistics and calculations were done under the R environment [23].

2.4 Health risk determination

Following the guidelines for evaluating chemical contaminants for use by fishery consulting services [24], the risk was characterized based on the evaluation of exposure limits and consumption of mercury and methyl-mercury. To determine the consumption risk, information was obtained from the survey on the following variables: (i) most consumed species in Providencia and Santa Catalina islands and (ii) frequency of consumption. Using the collected data from the surveys, and the oral reference dose for Hg (0.0005 mg Kg^{-1}-d^{-1}), the indices were calculated to determine the risk by consumption.

2.5 Single and multiple species exposure

The indices Single Species Exposure (*SSE*) and Multiple Species Exposure (*MSE*) were calculated to evaluate the risk represented by mercury due to the fish consumption frequency of one species or multiple species respectively, also the xenobiotic concentration in muscle, and the size of the consumed portion [24]. The *SSE* index was calculated by equation (2):

$$SSE = \frac{C_m * CR}{BW} \quad (2)$$

where *SSE*: individual exposure to xenobiotic due to ingesting fish (mg Kg^{-1}-d^{-1}); C_m: concentration of mercury in fish (mg Kg^{-1}); *CR*: consumption rate (Kg d^{-1}); *BW:* individual body weight (80 Kg). The value of *MSE* was calculated using equation (3):

$$MSE = \frac{\sum (C_{m,j} * CR_j * P_j)}{BW} \quad (3)$$

where *MSE*: individual exposure to xenobiotic by ingesting fishes of different species (mg Kg^{-1}-d^{-1}); $C_{m,j}$: concentration of mercury in the edible portion of fish species *j* (mg Kg^{-1}); CR_j: consumption rate of each fish species *j* (Kg d^{-1}); P_j: proportion of each fish species *j* in an individual diet (dimensionless); *BW* as in equation (2).

2.6 Target hazard quotient (THQ)

The *THQ* index is the ratio between measured concentration and oral reference dose and is weighted by the duration and frequency of exposure, ingested portion size, and body weight [24]. If THQ <1, it is unlikely that the exposed population might manifest adverse health effects due to the contaminant. The *THQ* risk estimation method used has been proposed previously [25, 26]:

$$THQ = \frac{EFr*ED*FIR*MC}{R_{fD}*BW*AT} \quad (4)$$

where *THQ*: Target Hazard Quotient; *EFr*: exposure frequency (365 d yr^{-1}); *ED*: exposure duration (70 yr); *FIR*: food ingestion rate (0.227 Kg $person^{-1}$-d^{-1}); *MC*: average concentration of metal in food (mg Kg^{-1}, on fresh weight basis and highest value measured); R_{fD}: oral reference dose for each metal (mg Kg^{-1}-d^{-1}) [24]; *BW*: average body weight of an adult (80 Kg); *AT*: average exposure time (365 d yr^{-1} times number of exposure years, assuming 70 yr).

3 | RESULTS AND DISCUSSION

The insights in consumption habits revealed that 56% of island inhabitants consume animal protein between 2 and 3 times a week, while 24% and 20% consume animal protein over 4 times a week or less than one time a week, respectively. The most important source of protein is poultry and pork due to their low price compared with beef and considering the incomes generated in the fish sales to fishermen

The survey identified the five species with the highest consumption preference, which are the Mutton snapper (*Lutjanus analis*), French Grunt (*Haemulon flavolineatum*), Blackfin Tuna (*Thunnus atlanticus*), Greater Amberjack (*Seriola dumerili*) and Great Barracuda (*Sphyraena barracuda*). The diet of the inhabitants is varied in fish products, identifying 27 species consumed, however, the frequency of consumption is low compared to other sources of animal protein.

Laboratory analysis for mercury for the five most consumed species reported concentrations of mercury between 0.957 to 1.904 mg Kg^{-1}, and concentrations of methyl-mercury between 0.15 to 1.3 mg Kg^{-1} (Figure 2). Some of these values exceed the maximum limits for Hg in fish established in Resolution 122 of 2012 of the Ministerio de Salud y Protección Social which dictates that the maximum limit of Hg for species with low trophic levels is 0.5 mg Kg-1 and 1 mg Kg-1 for high trophic level species. These species that exceed the maximum concentration allowed in national regulations, must have special attention because also they register a high preference for consumption among the inhabitants of the islands, they are the environmental health bioindicators that bond us to the ecosystem through a trophic link.

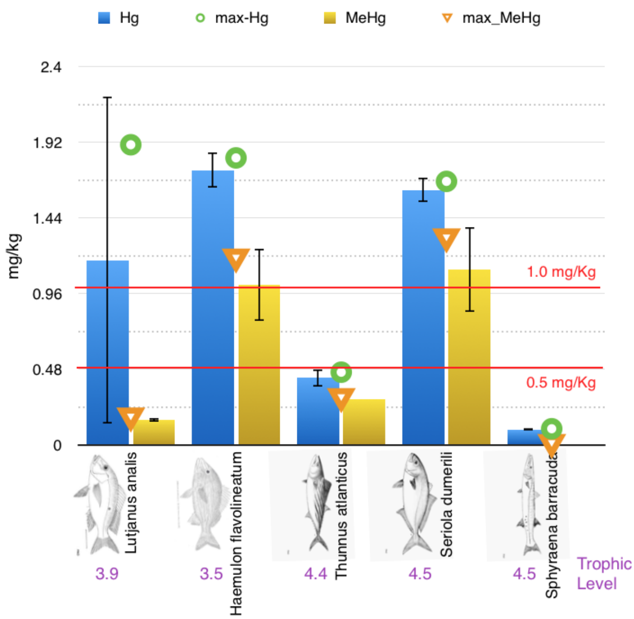

Figure 2. Mercury and methyl-mercury concentrations in the 5 most preferred species. Red line: maximum limit allowed by Resolution 122 of 2012 of the Ministerio de Salud y Protección Social (Hg = 0.5 mg Kg^{-1} for species with a low trophic level and 1 mg Kg^{-1} for species with a high trophic level). The error bars represent the standard deviation.

Considering the trophic level for each species as reported in FishBase, was observed that among the species with the highest consumption preference are predators with levels from 3.5 to 4.5 (Figure 2). *H. flavolineatum* was the only species with an intermediate trophic level of 3.5 but presented the highest mercury concentrations, this pattern is different from that mentioned in the literature, as there must find a higher concentration of metals at higher trophic levels [27, 28]. This bioaccumulation pattern could be explained considering: (i) the fishing spot from where the fishes came, a channel between Providencia and Santa Catalina islands, and (ii) the diet of this species since they feed on small crustaceans that are related to sediments [29].

During the 2019 Sea Flower Expedition, determinations of heavy metals were also made in sediments samples from fishing spots in a parallel unpublished study, which permits the authors to assume that the demersal feeding habits of *H. flavolineatum* have promoted a greater exposure to Hg through its diet, so reporting the highest mercury concentrations with mean values of 1.74 ± 0.11 Hg mg Kg^{-1}.

Whereas the other most consumed species have wider feeding habits feeding in the water column and open sea, where the environmental concentration of contaminants appears to be lower. However, has been evidence of a different pattern in the biomagnification of some xenobiotics through the food web because of the conditions of the study site [30]. This condition in Providencia and Santa Catalina would imply that practices to reduce exposure to contamination from fish consumption must be adjusted to each area's bioaccumulation and biomagnification patterns.

With the data collected in this assessment, the size of the organisms did not explain the differences in mercury concentrations in the individuals of the species included. The *H. flavolineatum* collected in this study were sub-adult individuals (10 cm-15 cm) with an average estimated age of <1 year [31, 32]; and the individuals of the other species analyzed were in their juvenile phase, *S. barracuda* registered average sizes from 60 cm to 65 cm and average estimated age of >2 years [33], *T. atlanticus* (45 cm-50 cm) approximate age >1 year [34]; *S. dumerili* (40 cm-45 cm) mean estimated age <1 year [35]; and *L. annalis* (30 cm-35 cm) mean estimated life <3 years [36]. The size of the fish, measured as weight and length, varies according to age, so it can be assumed that each organism presented different exposure times, this could explain the higher concentrations of mercury in individuals of *H. flavolineatum* due to exposure at higher concentrations of the contaminant and not to the exposure time.

Concentration data and consumption preferences were used to estimate the level of risk from exposure to Hg through the consumption of contaminated fish (Table 2). The *THQ* risk coefficient values >1 indicate carcinogenic risk [37], showing that for all fish species there is a high-risk level due to consumption, except for *S. barracuda*, a species that presents xenobiotic low concentrations and low consumption preference. For the values of the risk of exposure to a pollutant (*Em*) per person, the same pattern was observed as for *THQ* (Table 2). The value of the index of exposure to a pollutant by ingestion of multiple species of fish ($E_{m,j}$) is higher than the reference dose recommended by the EPA, generating concern about the potentially high risk to which the inhabitants of Providencia and Santa Catalina are exposed due to the consumption of fishes with high levels of mercury, because for most of the cases evaluated they exceed the recommended reference dose [38].

Species	(THQ)	SSE (mg Kg^{-1}-day^{-1})	MSE (mg Kg^{-1}-day^{-1})
Trophic level 3.5			
Haemulon flavolineatum	**4.341**	**0.000434**	0.00038
Trophic level 3.9			
Lutjanus analis	**7.188**	**0.000719**	0.00038
Trophic level 4.4			
Thunnus atlanticus	**1.010**	**0.000101**	0.00038
Trophic level 4.5			
Seriola dumerili	**3.654**	**0.000365**	0.00038
Sphyraena barracuda	0.216	0.000022	0.00038

Table 2. Risk indices for mercury ingestion. Values in bold represent risk from fish consumption.

Other relevant aspects may be: (i) the destination of the fish catches in the islands, in the case study, fish have high mercury values in muscle tissue, however, the surveys carried out indicated that fishing in the region is focused on supplying the national market and not local consumption. And (ii) the exposure of the most sensitive population, children, and pregnant mothers [39-41].

In Colombia, more than 40 years ago, the presence of heavy metals in different matrices (water, biota, fishes, sediments, soil) has been reported for all continental hydrographic basins of the country [42]. This contamination in continental areas is attributed principally to gold mining activities, estimating that the country has emitted up to 150 tons of Hg per year, which positions it as the largest per capita pollutant of Hg in the world [43]. However, the relationship of continental sources in coastal marine areas of the Colombian Caribbean has not been studied. Nevertheless, it is evident from the results of this study, the presence of mercury and exposure in insular areas of Colombia.

A review of the studies made in Colombia for the assessment of heavy metal concentrations in the muscle of the fishes for consumption showed the potential health risk and the affectation of food innocuity and food security [44]. In Providencia and Santa Catalina, the mercury values found in fish muscles exceed by 60% the reference value established by the World Health Organization of 0.5 mg Kg^{-1}, a potential risk not only for local consumers of fishery products but also for the consumers in the interior of the country [13].

4 I CONCLUSIONS

This study presents evidence of the potentially high risk due to exposure to mercury through the consumption of contaminated fish in the archipelago of San Andrés, Providencia and Santa Catalina since it exceeds the limit established by the EPA in 4 of the 5 species of fish included in the study.

The risk is higher in the species with the lowest trophic level; therefore, consumption recommendations must be based on the toxicokinetic characteristics of each context. French Grunt (*H. flavolineatum*) are organisms with a coastal ecological niche with epibenthic feeding habits that expose them to high Hg concentrations in sediments despite being the species with the lowest trophic level in this study.

The food sources of the local communities are influenced by social and economic factors, hence the responsibility for food security lies mainly with the regulatory entities at the regional and national levels, for this reason, environmental and food contamination must be monitored, legislation must also be strengthened to reduce the contaminants in the environment.

5 I DECLARATION OF COMPETING INTEREST

We declare that we have no significant competing interests, including financial or non-financial, professional, or personal interests interfering with the full and objective presentation of the work described in this manuscript.

6 I DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

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