Persistent Organic Pollutants (POPs) in fish biota of Guanabara Bay at Rio de Janeiro - Brazil

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Abstract. Fish is present in the Brazilian diet and represents an important participation in protein consumption. The use of highly toxic pesticides is still tolerated in Brazil despite being banned in many parts of the world. This study records traces of contamination by Persistent Organic Pollutants (POPs) at Guanabara Bay at Rio de Janeiro (Brazil). Our results indicate that local production and the widespread use of such chlorinated compounds in the past decades are being reflected on the edible fish tissue concentrations.

1 Introduction

Persistent Organic Pollutants (POPs) are organic chemical compounds that have physical and chemical properties in common and when released into the environment are highly resistant to degradation by biological, chemical or photolytic means. These substances, such as pesticides or industrial chemicals and industrial by-products, are intentionally or inadvertently produced and introduced into the environment by human action

POPs have been reported as an environmental concern issue since last decades. Nevertheless, their global threats are still difficult to access due to most research are focused on selected groups of contaminants while other groups remained unstudied [1]. The majority well established information are related to POPs which are regulated under the Stockholm convention since 2001 and represent a diverse group of organic substances, which are toxic, persistent, bioaccumulative and tend to long-range transport [2].

Urbanization and industrialization severely contribute to polluted hotspots and worldwide distribution of such pollutants. The most anthropogenically disturbed area along the Brazilian shoreline is Guanabara Bay, situated in Rio de Janeiro state [3]. This estuary is bordered by 12000 industries and four cities, with a total population of about 11 million people [4] Despite the anthropogenic pressure, Guanabara Bay supplies food and breeding grounds for several wildlife, which makes it an important fishing point to the local market and population.

Fish consumption in Brazil has grown considerably in recent years. Consumers are increasingly aware of these beneficial effects on human health, which also contributes to the continuous increase in fish consumption [5]. In addition, fish consumption has been promoted in order to boost the market for this sector in the country, adding a role to fish in the economic sector, since the fish chain is well consolidated and an important Brazilian agribusiness,

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contributing to a relevant GDP. each year and generating wealth for the country [6]. And with regard to the consumption of this food, consumer demand is linked to quality of life and health, since it is an important nutritional source [7].

In this work we tried to determine some target compounds classified as POPs, in three fish species with high level consumption, by gas chromatography (GC) coupled to electron capture negative ionization (ECNI) with a quadrupole mass spectrometer (MS).

2 Materials and methods

Fish sampling was carried out in Guanabara Bay, Rio de Janeiro, Brazil frozen and transported to the Federal University of Rio de Janeiro, Brazil. Only the muscle tissue was used. The methodology was proposed by Alonso et al. (2012) with modifications [8]. 50µL of the internal standard PCB 103 and 198 was added to freeze-dried Sardinella brasiliensis (n=9) (3g), Micropogonias furnieri (n=4) (6g) and (n=1) Mugil cephalus (n=1) (6g) samples. Soxhlet extraction was conducted for 8 hours with Hexane/Diclorometane (1:1).

Afterwards, the samples were concentrated under a gentle nitrogen flow. Then, clean-up procedure was performed into two steps. The first one took place in centrifuge tubes with sulfuric acid and then brought to centrifuge for 20 minutes, this operation was performed five times to obtain a translucent sample. The second step comprised a chromatographic column filled with 1 cm of sodium sulfate, 6 g of alumina (6%), 10g of acid silica (44%) and 1 cm of sodium sulfate eluted with 120 ml of hexane/dichloromethane (1:2). Finally, samples were concentrated under nitrogen, transferred into a vial, evaporated to dryness, and re-suspended to 100 µL of injection standard (TCMX 100 ppb).

Organohalogen pollutants were determined using a 7890N gas chromatograph equipped with a BD5 fused silica capillary column (60m x 0.25 mm x 0.25 µm film thickness) and coupled with a 5975C quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA). Operated in SIM mode for target compounds and in full scan mode, both with electron capture negative ionization (ECNI). Sample injection (1µL) was made at 260 ºC in pulsed hot splitless mode (4.0 min pulse and splitless time). Helium and methane were used as carrier (constant flow rate of 1.5 mL/min) and reaction gas, respectively. The oven temperature programmed was: 120 ºC for 4.2 min, 30 ºC/min to 200 ºC, 5 ºC/min to 275 ºC, 40 ºC/min to 300 ºC, held 10 min, and finally ramped at 10 ºC/min to 310 ºC and held 2 min. The temperatures of the transfer line, source and quadrupole were set at 300 ºC, 150 ºC and 150 ºC, respectively.

3 Results and discussion

The presence of 26 OCPs was investigated, including metabolites and isomers, namely: HCB, α-HCH, β-HCH, ᵞ-HCH, ᵟ-HCH, Heptachlor, cis-heptachlor epoxide, trans-heptachlor epoxide, Aldrin, Isodrin , Dieldrin, Endrin, Oxychlordane, cis-chlordane, trans-chlordane, α-endosulfan, β-endosulfan, o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD , o,p'-DDT, p,p'-DDT, DDMU, Methoxychlor and Mirex. All results are expressed in ng of OCP per gram of wet fish (ng/g p.u.) (Table 1).

Table 1. Comparison of the mean ± SD of the concentration of organochlorine pesticides (ng / g p.u.) in Sardine, croaker and mullet samples and P value using Kruskal Wallis test and subsequent Dunn's Test*.

	Sardinha n=20	Corvina n=19	Tainha n=16	P
HCB	0.016 ± 0.027[a]	0.005 ± 0.009[a]	0.006 ± 0.008[a]	0.1362
α-HCH	0.005 ± 0.007[a]	0.003 ± 0.006[a]	0.004 ± 0.007[a]	0.4847
β-HCH	<LOD	0.003 ± 0.010	<LOD	

γ-HCH	0.041 ± 0.122[a]	0.018 ± 0.035[a]	0.025 ± 0.051[a]	0.8098
Σ HCH	0.046 ± 0.128[a]	0.024 ± 0.051[a]	0.029 ± 0.056[a]	0.6456
Heptacloro	0.011 ± 0.039[a]	0.036 ± 0.076[a]	<LOD	0.0570
Aldrin	0.001 ± 0.003	<LOD	<LOD	
Dieldrin	<LOD	<LOD	0.005 ± 0.019	
cis-Heptacloro epóxido	<LOD	0.003 ± 0.011	<LOD	
cis-Clordano	0.004 ± 0.015[a]	0.015 ± 0.040[a]	0.007 ± 0.020[a]	0.3680
trans-Clordano	0.014 ± 0.027[a]	0.011 ± 0.021[a]	0.019 ± 0.043[a]	0.7575
Σ Clordano	0.018 ± 0.042[a]	0.026 ± 0.060[a]	0.025 ± 0.057[a]	0.9464
α-Endossulfam	0.000 ± 0.001[a]	0.001 ± 0.003[a]	0.002 ± 0.005[a]	0.2599
p,p'-DDE	0.766 ± 2.342[a]	0.695 ± 1.146[a]	0.286 ± 0.522[a]	0.3565
o,p'-DDE	0.429 ± 0.946[a]	0.018 ± 0.044[a]	0.343 ± 0.722[a]	0.1443
o,p'-DDD	0.068 ± 0.122[a]	0.014 ± 0.049[b]	<LOD	0.0224
p,p'-DDD	0.007 ± 0.031	<LOD	<LOD	
o,p'-DDT	0.459 ± 0.588[a]	0.005 ± 0.022[b]	0.008 ± 0.033[b]	<0.0001
p,p'-DDT	0.180 ± 0.400[a]	0.119 ± 0.199[a]	0.027 ± 0.090[a]	0.0665
DDMU	0.114 ± 0.096[a]	0.344 ± 0.492[a]	0.160 ± 0.217[a]	0.8049
Σ DDT	2.023 ± 4.526[a]	1.196 ± 1.953[a]	0.824 ± 1.244[a]	0.2284
Metoxicloro	4.462 ± 13.261[a]	6.162 ± 9.225[a]	1.955 ± 3.810[a]	0.2365
Σ OCP	6.576 ± 18.027[a]	7.454 ± 11.389[a]	2.846 ± 4.894[a]	0.1762

* Different letters on the same line differ statistically at the significance level of 5% ($p<0.05$), SD (standard deviation), <LOD (below the detection limit), Σ HCH (α-HCH + β-HCH + γ-HCH), Σ chlordane (cis-chlordane + trans-chlordane), Σ DDT (p,p'-DDE + o,p'-DDE + o,p'-DDD + p,p'-DDD + o,p'-DDD + DDT + p,p'-DDT + DDMU), σ OCP (sum of organochlorine pesticides).

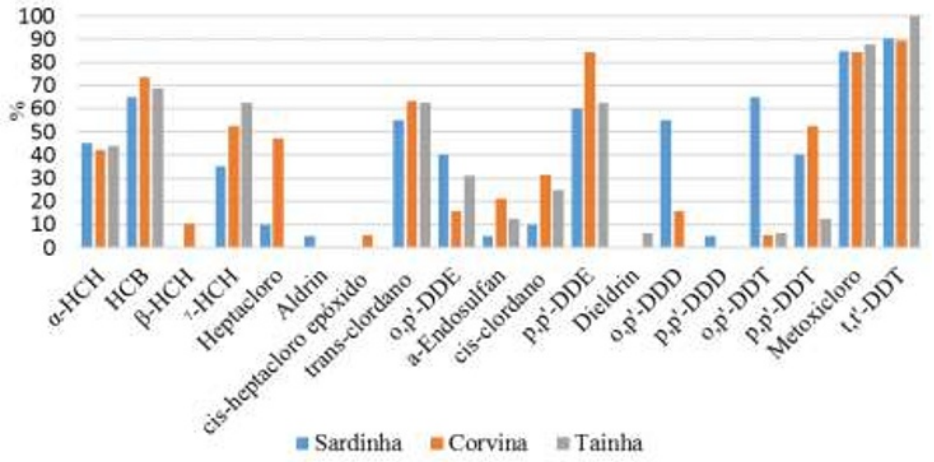

Fig. 1. Frequency of OCPs in true sardine, croaker and Mullet samples (t,t'-DDT = DDMU).

Regarding the recovery means of the method based on the concentration of PCB 103 and 198 calculated at the end of the experiment, they are within the range of analytical

acceptability which, according to the Directorate General Health and Consumer Protection of the European Commission (2000), can vary from 40 to 160% (Table 2).

Table 2. Mean ± SD recovery of PCB 103 and PCB 198 standards in fish samples analyzed for each species.

	Average Recovery (%)		
	Sardine-true	mullet	croaker
PCB 103	87 ± 49	100 ± 65	76 ± 54
PCB 198	124 ± 84	156 ± 123	88 ± 77

All evaluated fish samples contained at least one OCP and the total concentrations of these compounds (Σ OCP) ranged from 6.576 ng/g p.u., 7.454 ng/g p.u. and 2.846 ng/g p.u. for sardines, croaker and mullet, respectively. However, no significant difference was found between the sum concentration of these compounds among the three fish species studied. Among the 26 investigated substances, only 7 were not found in any of the samples, they are: $^{\delta}$-HCH, Isodrin, Endrin, Oxychlordane, trans-heptachlor epoxide, β-endosulfan and Mirex.

Thus, the profile contamination of POPs was mainly composed by polychlorinated biphenyls (PCB) followed by legacy organochlorine pesticides (OCP) and polybrominated diphenyl ethers (PBDE) both natural and anthropogenic compounds, according to the literature [1,9]. Moreover, the presence of some organohalogen compounds less commonly analyzed as the heptachlorobipyrrole (Q1) and pentachloroanisole (PCA) were found in all samples. Regarding these non-target compounds, Q1 has been previously reported in Guanabara Bay by Rosenfelder et al, 2012, in a study which they also document the presence of a new DDT metabolite in the local biota [1]. Pentachloroanisole is a common metabolite of pentachlorophenol (PCP) in fish, although it is not well documented. This product is only used in Brazil in wood protection since 1998, before that it was used as fungicide, algaecide and insecticide [10].

4 Conclusions

As depicted by our results, legacy and emerging POPs can be easily found in the high polluted Guanabara Bay. Data on the estimation of the toxicological risk of ingestion of OCPs through the consumption of fish from Guanabara Bay state that this consumption does not present a risk to human health at present. However, the presence of the less studied compounds as the natural orgahalogen pollutants highlights the lack of information regarding its worldwide distribution. This may represent a health risk hazard, primarily to fishermen and their families. We suggest that more research ought to be done in order to clarify this issue in the near future.

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