PERSISTENT ORGANIC POLLUTANTS IN MUSCLE OF FARMED NILE TILAPIA (*Oreochromis niloticus*) AND FEED FROM BRAZIL - PART II: PCB AND PAH

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Abstract

Tilapia raised on fish farm can be exposed through alimentary intake to persistent organic contaminants, such as PCB and PAH, due to a diet based in fish feed containing several ingredients which can content contaminants. The concentrations and congener's pattern of the PCB and PAH were determined in fish muscle and feed samples collected from different fish farms in Brazil (2 Intensive and 2 Super Intensive rearing systems). Results indicated that the ΣPCB mean concentrations in fish muscle and fish feed samples ranged from 114.7 to 300.4 pg g^{-1} and from 69.3 to 2310.4 pg g^{-1} wet weight basis (ww.), respectively. PCB were analyzed into three categories as follows: Indicator PCB (90.1% of total samples, majority found at SI systems), Non-ortho (were present in 0.5% of total samples) and Mono-ortho (9.4% of total samples). PAH was found in low levels, with Naphthalene and Phenanthrene the most found with 61% and 22% of total PAH concentrations. No correlations were found between PCB and PAH concentrations. Higher PCB concentrations in feed are corresponding to higher PCB concentrations in fish muscle, but not in case of PAH.

Key-words: Polychlorinated Biphenyls, Polycyclic Aromatic Hydrocarbons, environmental pollution, Tilapia, feed, aquaculture.

Introduction

Fish farming has developed due to the fact that the supply of traditional stock fish is declining because of overfishing and pollution. Furthermore, the demand for high-quality food is increasing due to population growth and health-related considerations. Most of Brazil has a tropical climate that will permit year around tilapia growth. The favorable conditions that make tilapia one of the most appropriate specie for fish farming are resistance to low concentrations of oxygen, great physical resistance to disease, fast growth, good utilization of artificial diets, and great fillet yield. The excellent quality of its firmly textured flesh, white color and few intramuscular bones make it an attractive, appetizing fish to consumers[1].

Contaminants are present in the fish oils and fish meals used in feed manufacture and some researchers speculate that all fish feeds contain measurable levels of some contaminants[2,3,4,5,6]. At a study on POPs eleven times more toxins in farmed fish was detected than in wild caught salmon. The farmed salmon content average was 36.6 ppb of PCB which was attributed to feed used in fish farms[3].

PAH are also a problem to fish. It was suggested that levels of PAH commonly found in many aquatic environments are an important risk factor for various health aspects of fish, such as adverse histopathologic and immunological responses in tilapia[7,8].

The objectives of the present study were to investigate the (1) levels of PAH and PCB in fish samples collected from four fish farms of Brazil; (2) comparison of levels of PAH and PCB in the super intensive and intensive systems; and (3) biotransfer and bioaccumulation of PAH and PCB from commercial fish feed.

Materials And Methods

Nile Tilapia (*Oreochromis niloticus*) weighted 58–81 g (juveniles) and 587-704g (adult) with lipid content ranged from 1.9–3.5%, were collected from four fish farms in different states of Brazil. Among these fish farms, 2 were of so called Super Intensive System (SI) and 2 of Intensive System (I) of culture. In each fish farm sixty fish muscle samples were collected in two raise phases. 30 samples of juveniles and 30 adults were prepared as a pool, where composite samples contained 6 specimens for each sample. Fish feed samples were collected for each raise phase and each fish farm. All samples (fish and feed) were kept below -28 ºC until analysis.

Extraction of pesticides was carried out with 10 g of freeze dried fish samples, using an Accelerated Solvent Extractor (ASE 200) device (Dionex, Sunnyvatem CA, USA) employing N-hexane/acetone (75/25 v/v) as eluent. The fat content in the extract was determined gravimetrically. For the analysis of PCB and PAH the clean-up procedures were applied to the samples according to Wang[9] adapted.

A GC-MS system consisted of an Agilent 5890 gas chromatography coupled to a Finnigan MAT 95 (Thermo) mass spectrometer was used for PCB and PAH determination. Selected ion monitoring (SIM) mode was employed for identification and quantification of 18 PCBs (PCBs were classified into three categories as follows: Indicator PCBs 28, 52, 101, 138, 153, 180; Non-ortho PCBs 77, 81, 126, 169 and Mono-ortho PCBs 105, 114, 118, 123, 156, 157, 167, 189 and 25 individual PAH compounds, which include that 16 identified as priority pollutants, by the United States Environmental Protection Agency (USEPA) due to their toxic, mutagenic and carcinogenic characteristics[10.]

The limits of detection were calculated on the basis of a signal to noise ratio of 6:1. For fish samples, the limit of detection were of 0.216 ± 0.153 pg g-1 (ww) for the PCB and 1.14 ± 1.02 pg g-1 (ww) for the PAH and recoveries of the 13C-labelled were 69 ± 14 % for the PCB and 113 ± 36 % for PAH. For feed samples, the limits of detection were of 0.591 ± 0.137 pg g-1 (ww) for PCB and 6.95 ± 3.83 pg g-1 (ww) for PAH and recoveries of the 13C-labelled were 78 ± 31 % for the PCB and 127 ± 33 % for PAH. The enforcing lab is operating a quality assurance system according to DIN EN ISO/IEC 17025 and is accredited for the analyses of PAH and PCB. The applied procedures were validated on the basis of internal reference materials and approved within international interlaboratory comparison studies.

The Graph Instat 3.0 (GraphPad Software Inc., San Diego, CA, USA) software package was used throughout this study to compare PCB and PAH concentrations in fish muscle and fish feed. One-way ANOVA was used to compare data from of fish muscle. In all cases, results were considered to be significant for $p < 0.05$ (Correlation Pearson).

Results and Discussions

The concentrations of PCB and PAH in muscle of Nile tilapia collected from four farms in Brazil are presented in Table 1.

Table 1 – Mean ± Standard Deviation values of PCB and PAH (pg g^{-1} ww.) in muscle of farmed Nile tilapia (*Oreochromis niloticus*) from Brazil

	Super Intensive 1		**Super Intensive 2**		**Intensive 1**		**Intensive 2**	
	Adult	**Juvenile**	**Adult**	**Juvenile**	**Adult**	**Juvenile**	**Adult**	**Juvenile**
PCBs								
PCB 28	61.2 ± 11.5	32.5 ± 5.3	48.9 ± 6.7	34.7 ± 10.4	51.8 ± 12.3	34.3 ± 4.5	30.5 ± 4.8	22.6 ± 2.5
PCB 52	61.2 ± 11.0	36.8 ± 6.3	50.7 ± 7.1	34.8 ± 9.5	50.4 ± 10.2	44.0 ± 12.0	31.5 ± 6.6	22.5 ± 4.9
PCB 101	43.1 ± 7.3	41.7 ± 7.6	30.5 ± 3.7	26.6 ± 3.9	30.1 ± 5.1	25.8 ± 7.9	16.8 ± 3.1	14.3 ± 2.4
PCB 138	33.3 ± 3.5	46.0 ± 7.2	12.2 ± 2.1	14.6 ± 1.4	16.3 ± 1.1	13.1 ± 3.4	10.3 ± 2.8	12.8 ± 2.6
PCB 153	56.8 ± 9.5	72.8 ± 10.6	19.7 ± 3.7	22.3 ± 2.1	23.5 ± 1.6	19.2 ± 4.5	15.6 ± 4.0	19.7 ± 2.9
PCB 180	16.6 ± 3.1	26.0 ± 4.5	6.0 ± 1.8	8.2 ± 1.0	9.1 ± 0.9	8.8 ± 2.4	8.4 ± 2.5	11.9 ± 3.2
Σ Indicator	272.3 ± 42.7	255.9 ± 39.8	168.1 ± 22.4	141.0 ± 21.8	181.2 ± 25.9	145.3 ± 20.3	113.1 ± 21.3	103.7 ± 14.4
Σ Non-ortho	1.4 ± 0.5	2.2 ± 0.2	1.1 ± 0.2	1.0 ± 0.1	1.4 ± 0.4	1.1 ± 0.3	1.2 ± 0.1	1.0 ± 0.1
Σ Mono-ortho	28.6 ± 6.2	33.1 ± 5.7	13.3 ± 2.4	15.1 ± 1.6	18.1 ± 3.1	15.2 ± 3.5	8.7 ± 1.8	10.6 ± 3.2
PAHs								
Naphthalene	5785.2 ± 1932.6	3727.6 ± 635.4	3266.2 ± 889.2	2698.6 ± 502.1	2866.4 ± 261.4	2111.4 ± 585.9	1487.0 ± 245.8	1364.4 ± 209.9
Acenaphthylene	124.4 ± 22.6	148.0 ± 94.5	94.5 ±101.3	101.3 ± 7.0	89.6 ± 70.6	70.6 ± 15.5	57.5 ± 10.2	49.6 ± 4.1
Acenaphthene	126.1 ± 50.4	111.5 ±108.7	108.7 ± 18.2	128.6 ± 25.9	103.5 ± 30.9	103.3 ± 35.7	68.9 ± 23.1	67.7 ± 19.1
Fluorene	291.0 ± 55.9	242.4 ± 265.4	265.4 ± 306.8	306.8 ± 39.9	252.9 ± 33.3	208.4 ± 61.9	170.8 ± 43.5	160.8 ± 34.5
Phenanthrene	1416.0 ± 192.2	1043.0 ± 145.7	1154.6 ± 146.9	896.2 ± 157.4	1049.4 ± 250.2	932.6 ± 186.3	750.2 ± 182.6	515.6 ± 83.5
Anthracene	105.1 ± 20.1	69.6 ± 11.9	80.1 ± 11.5	65.8 ± 8.2	67.3 ± 18.4	48.9 ± 16.7	48.0 ± 14.9	33.2 ± 7.7
Fluoranthene	171.8 ± 44.3	109.3 ± 17.1	103.7 ± 8.8	73.2 ± 7.9	84.1 ± 10.7	91.3 ± 34.9	70.7 ±31.8	49.2 ± 11.6
Pyrene	94.7 ± 24.2	59.9 ± 12.5	65.9 ± 6.2	41.6 ± 5.5	53.3 ± 19.4	49.7 ± 16.7	48.8 ± 20.1	28.8 ± 7.1
Benzo(g.h.i) perylene	132.4 ±170.0	10.9 ± 5.6	2.7 ± 2.1	80.0 ± 39.7	n.d.	n.d.	36.6 ± 8.7	16.5 ± 6.1

n.d. – not detectable

PCB were found in all samples, except PCB 81 that were found at SI1 and I2, and PCB 126 and 169, which were found at I1 and I2 fish farms. PCB congeners were found (wet weight) in the following rank 52 (42 pg g^{-1}) > 28 (40 pg g^{-1}) > 153 (31 pg g^{-1}) > 101 (29 pg g^{-1}) > 138 (20 pg g^{-1}) > 180 (12 ng/g) > 118 (10 pg g^{-1}) with a ΣPCB amounting to 192 pg g^{-1}. Only low levels of PCB were found in fish feed and can explain the low PCB content in fish flesh. In the present study PCB 52 was identified as the major contributor to the PCB concentrations in the fish samples different of Nile tilapia in Egypt, which present PCB 28 as the congener that contributed to more than 40% of the total of the PCB[11]. For all fish samples, the PCB profile was dominated by

the hexa-, tetra-, tri- to penta-CB isomers, which constituted 27, 22, 21 and 20 % of the total PCBs, respectively. Different profile of PCB was found for fish feed which predominant congeners were tetra-, penta-, tri- and hexa-chlorobiphenyls isomers, corresponding to 31, 30, 20 and 14%, respectively.

The concentrations of PAH in feed samples ranged from 4193 pg g^{-1} (ww.) to SI1, 4841 pg g^{-1} (ww.) to SI2, 4103 pg g^{-1} (ww.) to I1 and 2602 pg g^{-1} (ww.) to I2. For fish samples, Benzo(a)anthracene and Chrysene was detected just at SI1 SI2 and I2, as like Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene and Indeno(1,2,3-c,d)pyrene. For I1, none of these PAH were detected. Dibenzo(a,h)anthracene was found just in adults from SI1. The potency-weighted total concentrations of PAH in all muscle tissues were below the guideline value of 670 pg g^{-1} (ww.) for human consumption set by USEPA[10]. The present study indicates that tilapia fish have a low tendency to bioaccumulate PAH.

Recently, some authors have been explored the efficacy of replacing marine fish oil by vegetable ones to reduce the contamination of the aquaculture products[12]. It can be done without prejudice the performance and gain for tilapia culture[13]. However the possible contamination of vegetable oil with pesticides should not be ignored.

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