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Full Length Article

Distribution and Accumulation of Polychlorinated Biphenyls (PCB), Polycyclic Aromatic Hydrocarbons (PAHs) and Organo-chlorine Residues in the Muscle Tissue of *Labeo rohita*

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Abstract

The aquatic ecosystem of the River Ravi is facing serious challenges due to anthropogenic activities. The aim of this research was to determine the concentration of some pesticides, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), in muscle tissue of *Labeo rohita* from the River Ravi in order to determine the health risks associated with the eating of this popular fish. Gas chromatography was used for the detection of the first two groups of pollutants and gas chromatography/flame ionization detection was used for the latter. The results revealed that pesticides and polychlorinated biphenyls were below the recommended upper limits of these residues in freshwater fish, but that PAHs exceeded recommended levels. Mean concentrations of total hexachlorocyclohexanes, cyclodiene pesticides, as well as 1,1,1-trichloro-2,2-bischlorophenylethane and its metabolites (DDTs), were 0.092, 0.08 and 0.076 ng g^{-1}, respectively. The concentration of indicator-PCBs was 0.194 ng g^{-1}. The total PAHs detected were 0.4 µg g^{-1}. These pesticides were observed to have a pyrogenic source. The total toxicity equivalent concentration was determined as 0.03078 µg g^{-1}.

Keywords: Fish; Muscle; Pollutants; Gas chromatography; Pesticides; River Ravi

Introduction

Polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbon (PAHs) are stable lipophilic compounds that have the potential to accumulate and biomagnify through food chains (Vafeiadi *et al.,* 2014; Ghaeni *et al.,* 2015). PCBs and PAH are among the pollutants of highest concern in water bodies due to their long persistence the ecosystem (Liang *et al.*, 2007; Perugini *et al.*, 2007), and PAHs, in particular, are potentially very toxic to aquatic organisms. PAHs and PCBs are also recognized to be potentially carcinogenic (Zhang *et al.,* 2015; Zheng *et al.,* 2016).

The PAHs originate due to the partial burning of fossil fuels, natural organic matter, and wood (Xu *et al*., 2011; Frena *et al*., 2016). Due to their hydrophobicity, strong perseverance, and thorough transference potential, PAHs are extensively dispersed in the aquatic habitat as well as in the air (Halek *et al*., 2008), water (Li *et al.,* 2006a, b; Cheung *et al.,* 2007), soils (Zhu *et al.,* 2008; Yang *et al.,* 2013), sediments (Chen *et al.,* 2012), and organisms (Wang *et al.,* 2012; Li *et al.,* 2013). The PAH amalgam may also move between the various ecological niches.

The PAHs are widely distributed in water bodies and may be imbibed by suspended matter in water, and later deposited as sediments. Sediments with adsorbed PAHs are one of the main reasons of pollution in the freshwater ecosystem and aquatic fauna. Freshwater organisms not only intensify contaminants from sediments and water but also transposition of contaminants via the trophic levels of food chain (Zhang *et al.,* 2015). The fishes feed in different trophic levels and this feeding habit have a significant role in the accumulation of pollutants in various tissues (Abdolahpur *et al.,* 2014). PAH compounds are generated from vehicle exhaust emissions, low-temperature partial combustion of coal, oil, gas and debris, and other multiple sources (Qi *et al.,* 2014). Thus, PAHs are apportioned among various trophic levels of the food chain in intensification systems (Wang *et al.*, 2016). Consumption of polluted fish is an important health hazard to human population in developing countries (Fang *et al.,* 2009). Exposure to PAHs may cause various severe human health carcinogenic and genotoxic problems (Kamal *et al.,* 2015). PCBs are anthropogenic organic chemicals that leach into the aquatic environment from inadequately controlled harmful waste landfill sites; or from disposal of PCB-containing discharge into landfills; or in the emissions of poorly functioning waste incinerators (Liang *et al.,* 2007).

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The PAHs and PCBs have carcinogenic and teratogenic effects to freshwater animals and plants in the freshwater ecosystem. It is likely that assessment, distribution and accumulation of PAHs and PCBs in freshwater fish, may help to anticipate a possible danger to human health if any. River Ravi is the small river in the province of the Punjab, Pakistan and it originate from India. Its area from Shahdara Bridge to Ballouki Headworks (near Lahore, Pakistan) has badly influenced the attribute of water of river Ravi and eventually aquatic life (Mahboob *et al.*, 2015). According to our information, the concentration of PAHs and PCBs in the commercially important fish species, *Labeo rohita*, has not been properly investigated to know the status of these pesticides in the River Ravi in Pakistan. Therefore, the objective of this research was to assess the levels of few organo-chlorine, 7 PCB, and 16 PAHs, in *L. rohita* harvested at Head Ballouki on the River Ravi and to assess the health risk.

Materials and Methods

Sample Preparation

Twenty-one *Labeo rohita* fish divided into three groups were procured from Ballouki Head-works. The fish weighed in the range of 1000–1200 g and were tested in triplicate as a single unrepeated event for the estimation of pesticide residues. Fish muscles were separated after dissection, freeze-dried and ground to a fine powder. These muscle samples were assayed for lipid content by following a method described by AOAC (1995).

Detection and Quantification of Pesticides

A multi-residue method using gas chromatograph-mass spectrometry (Hewlett–Packard 6890 GCECD) with an electron capture detector was used to estimate the level of polychlorinated biphenyls, polycyclic aromatic hydrocarbons and six pesticides in the muscle samples. The method used in the present research work was exactly as previously reported (Mahboob *et al.*, 2015). “All chemicals were specifically for pesticide residue determination and were obtained from Sigma–Aldrich (USA). The purified water was procured from a Milli-Q water system (Millipore, Bedford, MA, USA). OCP, PAh and PCB standards were purchased from Dr. Ehrenstorfer (Germany). A stock solution of 16 priority PAHs and 7 PCBs were used (Hussein *et al.*, 2016).”

Standard quality assurance and quality control methods were adopted for sample analysis. Specifically, for each samples, a method blank, a spike blank and sample were accomplished. The spiked recoveries ranged from 92–108% with an 8–15% coefficient of variation for all OCP (organo-chlorine pesticides) compounds. For diagnostic accuracy and recovery efficiency, six analyses were carried out on PAH reference materials, HS-5 and 2974 (provided by the IAEA). The recovery efficiency in this study ranged between 92 and 111% with an 8–14% coefficient of variation. The limit of detection in this study was determined to be 0.0015 ng/g for PCBs, 0.0016 ng/g for pesticides and 0.01 μg/mL for each PAH.

Statistical Analysis

The results of these analyses of selected pesticide residue concentrations in fish was subjected to statistical analysis by Minitab software. ANOVA tested for potential differences in concentration levels in the studied pesticide residues.

Results

Pesticides

Pesticides were detected in *L. rohita* in the following order of concentration: hexachlorocyclohexanes (α, β and γ isomers) > cyclodienes ≈ DDT and its metabolites. The total hexachlorocyclohexane (HCH) concentration was found to be 0.409 ng g^{-1} (Table 1). Alpha isomers were predominant at about 45.34%. The concentration of lindane and γ-isomer was recorded as 0.119 and 0.105 ng/g, respectively. The total concentration of cyclodiene pesticides (Aldrin, dieldrin and endrin) was observed to be 1.005 ng g^{-1}. Eendrin was most abundant (46.77 %) among the cyclodienes, followed by dieldrin (30.75%) and aldrin (21.49%) (Table 1).

The total concentration of DDT and metabolites was recorded as 1.792 ng g^{-1}. The major components were DDT, 1,1-dichloro-2,2-bischlorophenylethylene (DDE) and 1,1-dichloro-2,2-bischlorophenylethane (DDD). The percentage contribution of individual DDTs was as follows: p,p-DDT (18.36%), p,p-DDE (12.61%), p,p-DDD (17.52%), o,p-DDT (17.24%), o,p-DDD (22.76%) and o,p-DDE (11.43%).

Polychlorinated Biphenyls

The level of selected-PCBs (sum of PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180) that was detected in muscle tissue of *L. rohita* was 1.828 ng g^{-1} (Table 2). The average concentration of PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180 was detected in muscle tissue of *L. rohita* as 0.242, 0.106, 0.227, 0.331, 0.228, 0.222 and 0.472 ng/g, respectively (Table 2). The highest chlorinated PCB 180 was the most abundant congener, representing about one quarter of the concentration (25.82%), while PCB 52 was the least abundant (5.8%).

Table 1: Mean concentration (ng/g), percentage, and maximum residue level (ng/g) of some organo-chlorine pesticides in *L. rohita* collected from Head Ballouki, River Ravi

Pesticide group	Pesticide	Mean ± SD	Within the pesticide group (%)	Maximum level
HCH	α HCH	0.185±0.001	45.34	10*
	β HCH	0.105±0.001	25.73	
	γ HCH (Lindane)	0.119±0.004	29.17	1000*
	Total HCH	0.409±0.006		
Cyclodiene	Aldrin	0.216±0.002	21.49	100*
	Dieldrin	0.309±0.003	30.75	
	Endrin	0.470±0.003	46.77	ND **
	Total cyclodiene	1.005±0.008		
DDT and metabolites	o,p- DDD	0.408±0.002	22.76	ND **
	p,p-DDD	0.314±0.001	17.52	ND **
	o,p- DDE	0.205±0.001	11.43	ND **
	p,p-DDE	0.226±0.004	12.61	ND **
	o,p- DDT	0.309±0.001	17.24	ND **
	p,p-DDT	0.329±0.005	18.36	ND **
	Total DDT	1.792±0.015		1000 *
Total Pesticide		∑= 3.206		

*Australia New Zealand Food Standards Code Maximum Residue Limits 1.4.2 (2015); **ND = limit not determined in any national or international standard

Table 2: Mean concentration (ng g^{-1}), percentage, and maximum residue level (ng g^{-1}) of indicator-PCBs congeners in *L. rohita* collected from Head Ballouki, River Ravi

PCB congener	Mean ± SD	Within total PCB concentration (%)	Maximum level
PCB 28	0.242±0.006	13.23	ND*
PCB 52	0.106±0.002	5.80	ND*
PCB 101	0.227±0.003	12.42	ND*
PCB 118	0.331±0.001	18.10	ND*
PCB 138	0.228±0.004	12.47	ND*
PCB 153	0.222±0.002	12.14	ND*
PCB 180	0.472±0.003	25.82	ND*
Total PCBs	∑= 1.828±0.021		75 (EU)**

*ND = limit not determined for crustaceans in any national or international standard** European Commission (2011a, b)

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) were assessed in the *L. rohita* collected from Head Ballouki on the River Ravi. In this study, the total PAHs content detected in *L. rohita* was 4.725 µg/g. The individual contributions were acenaphthene (10.05), acenapthylene (3.47%), anthracene (2.3%), as benzo (a) anthracene (12.93%), benzophenanthrene (4.42%), benzo (b, k, g, h and I 13.3%) and dibenzo (a, h) anthracene (12.59%). Few PAHs listed on the EPA as poisonous chemicals (USEPA, 2012) like acenaphthene (0.475 µg g^{-1}), acenaphthylene (0.164 µg/g), anthracene (0.109 µg/g), benzo (g, h, i) perylene (4.35 %), phenanthrene (4.3%) and pyrene (3.51%) were recorded in this study (Table 3).

Discussion

Organo-chlorine pesticides (OCP) were present in extremely low concentration, these findings also indicate a continuous input of DDTs, and since the parent compounds are present in higher percentages than the metabolites, namely DDE and DDD. These findings were in accordance with those of Akhtar *et al.* (2014), who mentioned a fresh input of DDTs to the water body, based on DDE concentrations comprising between 1-50% of all DDT forms (Khaled *et al*., 2004; Akhtar *et al.*, 2014). The results of this investigation, however, contradict the findings of Newsome and Andrews (1993), who reported DDEs to be the major element of this group of pesticides; and attributed this to the continuous breakdown of DDT without a new influx of water bodies.

According to the Codex, the MRL for pesticides in freshwater fish is 10 ng/g for HCHs other than lindane, which has a limit of 100 ng/g. In addition, the Codex has established an MRL of 100 ng/g for the total of aldrin/dieldrin, 300 ng/g and 500 ng/g for DDT and its metabolites, respectively (USEPA, 2008). The pesticide residues in this study were below these maximum residue limits (MRLs) suggested by various agencies (Table 1). This could be due to the ban placed on the use of some of these pesticides in Pakistan and other countries of this region (Mahboob *et al*., 2011, 2015). Our results are also in conformity with the results of Akhtar *et al.* (2012). The results of this study indicated that these toxicants might be initiated from various regional atmospheric flows and then transported into this river. The higher pesticide concentrations reported in this study could be due to drainage and run off, with the pesticide residues coming from surrounding agricultural land into the River Ravi.

Table 3: Mean concentration (μg g^{-1}), percentage, and maximum residue level (μg g^{-1}) of some PAHs in *L. rohita* collected from Head Ballouki, River Ravi

PAHs	Mean ± SD	Within total PAHs (%)	Maximum level
Acenaphthene	0.475±0.002	10.05	ND*
Acenaphthylene	0.164±0.003	3.47	ND*
Anthracene	0.109±0.001	2.30	ND
Benzo(a)pyrene..	0.208±0.003	4.40	0.002 BaP**
Benzo(a)anthracene..	0.611±0.005	12.93	0.012 PAH4**
Benzo(a)phenanthrene (Chrysene)	0.209±0.002	4.42	
Benzo(b)fluoranthene..	0.305±0.004	6.45	
Benzo(k)fluoranthene	0.118±0.003	2.50	ND*
Benzo(g,h,i)perylene	0.206±0.002	4.35	ND*
Dibenzo (a,h)anthracene	0.595±0.003	12.59	ND*
Fluoranthene	0.352±0.002	7.44	ND*
Fluorene	0.429±0.004	9.07	ND*
Indeno(1,2,3) pyrene	0.257±0.005	5.43	ND*
Naphthalene	0.318±0.002	6.73	ND*
Phenanthrene	0.203±0.003	4.30	ND*
Pyrene	0.166±0.002	3.51	ND*
Total PAH	∑= 4.725±0.018		ND*

*ND = limit not determined for crustaceans in any national or international standard; **EU Commission (2011)

The HCH isomers were also assessed by Topi *et al.* (2006), who reported quite a high concentration of the sum of a, b and d-HCH in *Salmo letnica* (28.33 mg/kg w/w), and little low concentration in *Cyprinus carpio* (15.69 mg/kg w/w), although this is exceeded compared to this report. Mahboob *et al.* (2015) also reported a higher content of OCPs in *Catla catla*, exceeding the MRL suggested by international organizations. Lastly, lindane concentration was measured in the fish species caught from Head Ballouki on the River Ravi, showing levels higher than the FAO/WHO permissible limits. Elevated concentrations of p, p-DDT were detected in *L. rohita*, and this was attributed to the current use of DDT in Asian countries (Tanabe *et al.*, 2000). There, such pollution has been shown to cause damage to aquatic animals and to pose a serious health danger to consumers (Galindo-Royes *et al.*, 1999). This might be because of use of DDT, for health safety as a spray to control the malaria vector and antifouling paints (Quinn *et al.*, 2011). HCHs has an even distribution in the habitat with some spatial variation compared to DDTs (Topi *et al.*, 2005).

A highest chlorinated PCB 180 was the most abundant congener, representing about one quarter of the concentration (25.82%), while PCB 52 was the least abundant (5.8%). Low levels of PCBs were also comparable to the results of two studies carried out in China, which found that PCB level in aquatic organisms varied from N.D. to 23 ng/g, and from 0.83 to 11.4 ng/g (Chen *et al.*, 2002; Van Al *et al.*, 2012). The pesticide residues present in the aquatic habitat are reported to accumulate in muscle and augment in the ecological chain as a possible threat to human health (Sobek *et al.*, 2010). Moreover, the results of this study can be explained by feeding habit of *L. rohita*, which is column feeder and can partially feed on sediment-associated material, can bear relatively more pollutant loads compared to *C. catla*. The European Union (EU, 2011a) has specified the MRL to be 75 ng/g for the indicator-PCBs in fish and crustaceans, while FDA (1990) has specified it to be 2000 ng/g for total PCBs. According to EU (2011), therefore, the *L. rohita* from the River Ravi were found to be contaminated with PCBs (Table 2).

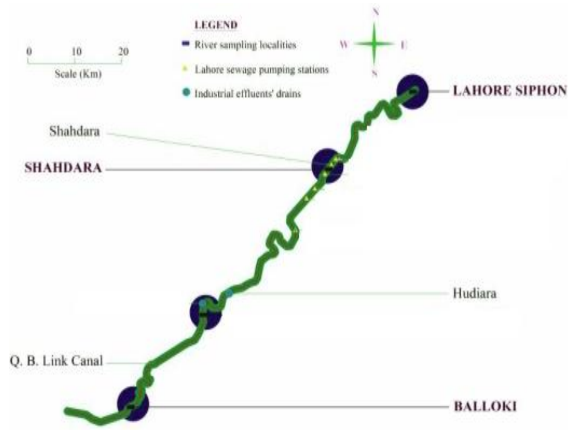

Fig. 1: Map of the Ravi River of study area (source: Shakir and Qazi, 2013)

In order to protect humans, MRLs are set for PAHs in few fatty-foods and in smoked food (EC, 2006). EU Scientific Committee (2008) considered benzo [a] pyrene as an important marker for PAHs in food, and suggested an MRL of 5 μg/kg wet weight. EFSA (2008) proposed a total concentration of 4 PAHs (benzo [a] pyrene, Benz [a] anthracene, benzo [b] fluoranthene and chrysene) as an excellent better benchmark of the presence of PAHs in food, whilst retaining the highest concentration for benzo (a) pyrene as a standard to compare between earlier and future studies. According to EU regulation No.835/2011 (EU, 2011b), the suggested MRLs for PAHs in addition to the MRL for benzo [a] pyrene are 30 and 5 μg/kg, respectively.

These limits were further reduced from 1st September 2014 to 12 μg/kg for PAH4 and 2 μg/kg for benzo [a] pyrene (EU, 2011b).

In this study, the *L. rohita* collected from Head Ballouki on the River Ravi was found to be contaminated with PAHs, cross the MRL for benzo [a] pyrene by 3.5 times and for PAH4 by 10 times, respectively (Table 3). This may be because of combustion of fossil fuel from various industries situated in the region and untreated discharge from various industries in the vicinity of this river. Salem *et al.* (2014) also reported that total PAHs varied between 0.74 and 456 ng/g, with an average value of 33 ng/g.

L. rohita is valued as a source of vitamin D and omega-6 fatty acids (Mahboob, 1992), and is the most popular fish among consumers in India, Pakistan, Bangladesh and Nepal. Jaward *et al*. (2012) mentioned that total PAHs ranged from 0.036 to 0.5 μg/g, with phenanthrene being the predominant PAH analogue (25%) detected, followed by pyrene and fluorine. The presented results were in line with the total PAHs in *L. rohita* tissues (Neser *et al.*, 2012).

Conclusion

The concentration of pesticides, PCBs and PAHs in *L. rohita* collected from Head Ballouki were below the maximum permissible residue level in fish. PAHs, however, exceeded the MRL values set by various agencies. These PAHs were observed to be from pyrogenic and pterogenic sources. Estimation of the cancer risk posed by the eating of *L. rohita* polluted with PAH.

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