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Organochlorine Pesticides, Polychlorinated Biphenyls and Heavy Metals Residues in Myctophids off South West Coast of India

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Abstract

Though myctophids are widely distributed in the Indian oceanic waters and contribute to a large proportion of the deep-sea fish biomass, the information regarding the presence of lipophilic chemical contaminants and heavy metals in these lipid-rich lantern fishes are relatively scanty. Analyses on the occurrence of heavy metals, organochlorine (OCPs) and polychlorobiphenyls (PCBs) in myctophids from Indian waters showed presence of organochlorine pesticides in *Diaphus watasei, Diaphus luetkeni* and *Myctophum obtusirostre*. The prominent pesticides detected in the fish tissue include endrin, dieldrin, heptachlor epoxide, α-BHC and heptachlor. Dieldrin and heptachlor epoxide were detected in *M. obtusirostre* while DDT isomers in *D. luetkeni*. The total PCB levels followed the order *D. luetkeni*> *D. watasei*>*M. obtusirostre* and 2, 2′, 3′, 4, 5 – pentachlorobiphenyl and 2, 3, 3′, 4′, 6-pentachlorobiphenyl. Trace metals (Fe, Zn, Cu and Mn) Composition is comparable with other fish species reported and followed the order *D. luetkeni*> *M. obtusirostre*> *D. watasei*. Results showed that POPs and heavy metal levels in myctophid species studied were much below the regulatory limits.

Keywords: Myctophids, organochlorine pesticides, polychlorinated biphenyls, heavy metals

Introduction

Persistent organic pollutants (POPs) are lipophilic in nature and resistant to degradation, leading to bioaccumulation and biomagnification along the food web leading to harmful health hazards (Skarphedinsdottir et al., 2009). Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs) are the banned organic pollutants responsible for food hazards, having reported specific roles in the disruption of endocrine glands, reproductive systems and immune systems (Ropstad et al., 2006, Yu et al., 2011; Lindenau & Fischer, 1996). Though the route of entry into a living system is diverse, the oral intake is reported to be a primary cause for their hazardous effect. The exposure is manifested several folds in humans, being so due to their presence at the top of the ecological pyramid. In the system, the catabolism of DDT to DDE via dehydrochlorination has a key role in DDT detoxification and the rate of reaction is rather slow (Katagi, 2010). Once in the marine environment, POPs get adsorbed to the detritus and marine snow and are eventually carried to the deep-sea where they biomagnify in the food web (Froescheis et al., 2000). Their lipophilicity and persistence allows them to be readily carried to long distances and tend to accumulate in living organisms. They are known to be responsible for carcinogenic, mutagenic and teratogenic effects (Biego et al., 2010). Deep-sea has been characterized as a sink for PCBs and other POPs, reflected by higher concentrations in deep-sea fish compared to fish from surface waters (Mormede & Davies, 2003).

Bioaccumulation of trace and heavy metals in fish is dependent on both the bioavailable concentration and species specific physiological and ecological characteristics. Metal distribution between the different tissues within an organism depends on the mode of exposure and can serve as a pollution indicator (Maheswari et al., 2006). Heavy metal (HM) concentration in fish tissues reflects past or present exposure and incorporation occurs mainly through the gills, skin or by food (Terra et al., 2008).

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The accumulation of metals in fish depends on an equilibrium between absorption and depuration rates (Mansour & Sidky, 2002; Karadede et al., 2004) and thus may reflect localized bioavailability of these substances. Mesopelagic lantern fishes, sampled from South Atlantic Ocean were found to have significantly larger concentrations of PBDEs in their tissues suggesting that higher brominated congeners of PBDEs, added to plastics as flame-retardants, are indicative of plastic contamination in the marine environment (Rochman et al., 2014). Myctophids are the key members in the mesopelagic region playing an important role in oceanic energy dynamics as well as food source for a number of aquatic lives and are considered to be the resources for the future. Though extensive research on chemical contaminant profiling of myctophids are available in Atlantic and Pacific oceans, the toxic residue status in myctophid fish species available in Indian waters are relatively scanty. It is in this background, an attempt was made to examine the myctophid fish species available in Indian waters for the presence of POPs and heavy metal residues.

Materials and Methods

Lantern fishes *D. watasei, D. luetkeni* and *Myctophum obtusirostre* were collected from commercial deep sea shrimp trawlers operated off Quilon, (8º 31' N - 9º 07' N & 75º 49' E - 75º 68' E) during March 2009. *D. watasei* (average size 13±0.6 cm and weight 22±1.2 g) was filleted, deskinned and homogenized for the analyses. *D. luetkeni* (average size 8.5±0.4 cm and weight 6.5±0.8 g) and *M. obtusirostre* (average size 8±0.3 cm and weight 5±0.6 g) were homogenized as whole due to their small size. The homogenized fish samples were analyzed for environmental pollutants namely OCPs, PCBs and HM.

Deionised (Milli – Q) water was used for sample preparation for analysis of heavy metals, while HPLC grade acetonitrile and hexane, GR grade petroleum benzene, diethyl ether, anhydrous sodium sulphate, sodium chloride and florisil (Sigma) were used for organochlorine pesticides and polychlorinated biphenyls. The organochlorine pesticide mix (Supelco, 47557 – U) was used for quantitative analysis and included isomers of DDT, BHC, heptachlor, dieldrin and endrin. The individual standards of PCBs (Dr. Ehrenstorfer GmbH) which included 24 PCBs of tri, tetra, penta, heaxa, hepta and octa chlorinated biphenyls.

Homogenized fish samples were digested with nitric acid/sulphuric acid (1:1), filtered and made up to 50 ml with Deionised water. The samples were quantified by an Atomic Absorption Spectrophotometer (Varian 220, USA) in an air-acetylene flame using appropriate hollow cathode lamps as per the validated methods and standard conditions. The metals studied were Zinc (Zn), Copper (Cu), Iron (Fe), Cobalt (Co), Cadmium (Cd), Lead (Pb), Manganese (Mn), Chromium (Cr) and Lithium (Li).

For determination of OCPs and PCBs, homogenized fish samples were extracted with petroleum benzene (60-80°C) and partitioned with acetonitrile, followed by solvent exchange with petroleum benzene (USFDA, 1999). The extract was cleaned up with florisil using petroleum benzene and diethyl ether and finally made up to 10 ml with hexane. The pollutants were quantitated by using a gas chromatograph (Varian CP 3800) with a radioactive Ni 63 Electron Capture Detector (ECD). Injection was done in split less mode and nitrogen was used as make up gas for both OCPs and PCBs. Samples (1 μL) were injected on to CP Sil 8CB capillary column (30 m x 0.25 mm x 0.25 μm) with injector and detector temperatures were 275 and 300°C, respectively. The oven temperature started at 170C, increased at a rate of 4° to 250°C and held for 10 min. For OCP residues, initial oven temperature of 180°C increased to 250°C at a rate of 5°C min^{-1} and held for 18 min followed by an increase to 280°C at a rate of 25°C min^{-1} and held for 5 min. Nitrogen was the carrier gas at a flow rate of 0.7 ml min^{-1} for and the detector temperature was set at 300°C.

A series of standards of heavy metals, organochlorine pesticides and polychlorinated biphenyls were spiked into the matrix and the noise to signal ratio 10 obtained was taken as method detection limit. The linear range for OCPs was found to be 10, 20, 30, 40 and 50 μg L^{-1} and that of PCBs were 20, 40, 60, 80 and 100 μg L^{-1}. 10 replicates of analysis were carried out for the determination of MDL. The recovery at the MDL was done in triplicates and given in Table 1.

Results and Discussion

Analyses of OCPs content in myctophid fish species (Table 2) indicated that endrin was the major pesticide 12-16 ng g^{-1}) detected in the sample followed by heptachlor, p, p'-DDT, α-BHC and o, p'-DDT. Dieldrin was detected at a level of

13.78 ng g^{-1} in *M. obtusirostre*. Among the isomers of DDTs heptachlor showed wide variation among the 3 species, while in the case of BHCs no significant variation was noticed. Total OCPs were in the order *M. obtusirostre* > *D. luetkeni* > *D. watasei*, while for PCBs the order was *M. obtusirostre* > *D. Watasei* > *D. luetkeni*. Concentration of PCBs in *D. luetkeni* was negligible compared to other species (Table 3). The PCBs, 2,2′,3′,4,5-pentachlorobiphenyl (47.12 ng g^{-1}) and 2,3,3′,4′,6- pentachlorobiphenyl (26.41 ng g^{-1}) predominated among the PCBs. Even though myctophids are fatty fishes and the POPS tend to accumulate in the lipid portion, no positive correlation was observed with contaminants in the present study. This present observation concurs with the earlier reports that the levels of total halogenated compounds were comparatively higher in migratory species followed by semi-migratory and non-migratory, except in *D. theta*, living in the mesopelagic and other deep-sea fishes (Takashi et al., 2010, de Brito et al., 2002; Takashi et al., 2000). Studies on the distribution of OCPs and PCBs in the deep-sea fishes reported higher levels in males than females which could be related to the ability of the females to eliminate contaminants through egg production (Mormede & Davies, 2003). There are reports on the accumulation of OCPs in the edible portion of fish in marine, brackish water and freshwater in India (Sankar et al., 2006) respectively and BHC and heptachlor epoxide formed the major share of OCPs in marine fish while BHCs contributed to the major share in freshwater and brackish water fish. Presence of persistent pollutants like polychlorinated biphenyls (PCBs), hexachlorocyclohexane (HCH) isomers, and dichlorodiphenyltrichloroethane (DDT) and its metabolites in seawater and sediment samples collected from six locations along the east coast of India were reported by Babu Rajendran et al. (2005) and tropical coastal waters of India (Sarkar et al., 2008).

Results of heavy metal profiling of myctophids (Table 4) showed that myctophids were a good source of Fe, Zn, Cu and Mn (Fe > Zn > Cu > Mn).

Table 1. Recovery (%) of organochlorine pesticides, polychlorinated biphenyls and trace metals in fish matrix

Pesticides*				Trace metal**			
	Spiked concentration (μg g-1)	Recovered concentration (μg g-1)	Recovery (%)	Element	Spiked concentration (μg g-1)	Recovered concentration (μg g-1)	Recovery (%)
α-BHC	2.5	2.16	86.4	Zn	0.500	0.445	89
β-BHC	2.5	2.23	89.2	Cu	0.500	0.438	87.6
γ-BHC	2.5	2.25	90	Pb	0.750	0.675	90
Heptachlor	2.5	2.14	85.6	Cd	0.250	0.230	92
Aldrin	2.5	2.08	83.2	Mn	0.250	0.213	85.2
Heptachlor Epoxide	2.5	2.06	82.4	Cr	0.250	0.220	88
Dieldrin	2.5	2.04	81.6	Co	1.00	0.850	85
p,p′ - DDE	2.5	2.20	88.0	Fe	0.250	0.223	89.2
o,p′ - DDD	2.5	2.18	87.2	Li	2.50	2.124	85
Endrin	2.5	2.16	86.4				
p,p′ - DDD	2.5	2.22	88.8				
o,p′ - DDT	2.5	2.27	90.8				
p,p′ - DDT	2.5	2.26	90.4				
Polychlorinated biphenyls	2.5	4.00 – 4.25	80 - 85				

*Sample size 50 g; spike concentration 2.5 μg g^{-1}; **Sample size 5 g

Table 2. Organochlorine pesticides content in myctophids (ng g^{-1} wet weight)

Pesticides	*D. watasei*	*D. luetkeni*	*M. Obtusirostre*	MDL
α-BHC	BDL	1.47±0.17*	BDL	2.5 ng g^{-1}
β-BHC	BDL	2.53±0.22	BDL	2.5 ng g^{-1}
γ-BHC	4.38±0.97	1.07±0.02*	6.38±0.16	2.5 ng g^{-1}
Heptachlor	5.56±0.69	1.17±0.07*	6.65±0.88	2.5 ng g^{-1}
Aldrin	BDL	BDL	BDL	2.5 ng g^{-1}
Heptachlor Epoxide	1.63±0.28*	0.51±0.11*	12.67±1.41	2.5 ng g^{-1}
Dieldrin	BDL	BDL	13.78±0.64	2.5 ng g^{-1}
p,p′ - DDE	BDL	BDL	BDL	2.5 ng g^{-1}
o,p′ - DDD	BDL	BDL	2.98±0.16	2.5 ng g^{-1}
Endrin	15.28±1.22	12.40±0.25	16.35±0.50	2.5 ng g^{-1}
p,p′ - DDD	BDL	8.76±0.93	BDL	2.5 ng g^{-1}
o,p′ - DDT	BDL	5.91±0.86	BDL	2.5 ng g^{-1}
p,p′ - DDT	BDL	0.67±0.22*	BDL	2.5 ng g^{-1}
Σ BHCs	4.38	5.07	6.38	—
Σ DDTs	—	15.34	2.98	—
Σ HEPs	7.19	1.68	19.32	—
Σ OCs	26.94	34.49	45.78	—

* BDL: Below Detection Level, ND: Not detected and MDL: Method Detection Limit.

Concentrations of these elements in myctophids were in the order *D. luetkeni* > *M. obtusirostre* > *D. watasei* except for Cu. Cu is an essential part of several enzymes and it is necessary for the synthesis of haemoglobin. No Cu deficiencies in human adults have been reported but, in infants, anemia and hypoproteinemia are reported (Underwood, 1977). Interestingly the variation in the concentration of Zn, an essential trace element required in metabolic process in both plant and animals, varied widely between and within the species of myctophids. A deficiency of Zn is marked by retarded growth, loss of taste and hypogonadism, leading to decreased fertility (NAS-NRC, 1974). Fe content was comparatively less for *D. watasei* and almost same concentration was obtained for *D. luetkeni and M. obtusirostre.* Deficiency of Fe leads to anemia and is associated with poor neurological development in children (Killip et al., 2007). Manganese content was below detectable limit in *D. watasei* while in *M. obtusirostre* and *D. luetkeni* showed trace levels (2.7 μg g^{-1}). Manganese is an essential element for both animals and plants and deficiencies result in severe skeletal and reproductive abnormalities in mammals. It is widely distributed throughout the body with little variation and does not accumulate with age (NAS-NRC, 1977). The toxic heavy metals Pb and Cd were below detectable level in the species studied indicating the safety of the fish for human consumption. There are reports available indicating the presence of heavy metals in the edible portion of molluscs, crustaceans, fish and fish products collected from fish markets in Cochin, Kerala, India but the levels were found to be within regulatory limits (Sankar et al., 2006; Sivaperumal et al., 2007).

Investigations by Mormede & Davies (2001) have shown that fish caught from 400 to 1150 m depth on the continental slope of Rockall Trough west of Scotland, exhibited the presence of arsenic, cadmium, copper, lead in the range 1.25 to 8.63, 0.002 to 0.034, 0.12 to 0.29 and 0.002 to 0.009 mg kg^{-1} wet weight, respectively, especially with higher accumulated concentrations in hepatic tissue. Reports by

Table 3. Polychlorinated biphenyls content in myctophids (ng g^{-1} wet weight)

Pesticides	*D. watasei*	*D. luetkeni*	*M. obtusirostre*	MDL
24′5 Tricholorobiphenyl	ND	1.60±0.26*	ND	5 ng g^{-1}
2′34 Tricholorobiphenyl	ND	2.72±0.27*	ND	5 ng g^{-1}
22′45' Tetracholorobiphenyl	ND	0.44±0.01*	ND	5 ng g^{-1}
233′4′ Tetracholorobiphenyl	ND	ND	ND	5 ng g^{-1}
2344' Tetracholorobiphenyl	ND	ND	ND	5 ng g^{-1}
23′4′5 Tetracholorobiphenyl	ND	ND	ND	5 ng g^{-1}
244′5 Tetracholorobiphenyl	ND	ND	ND	5 ng g^{-1}
22′345' Pentacholorobiphenyl	ND	ND	47.12±1.78	5 ng g^{-1}
22′35′6 Pentacholorobiphenyl	ND	ND	ND	5 ng g^{-1}
22′3′45 Pentacholorobiphenyl	ND	ND	2.87±0.09*	5 ng g^{-1}
22′44′5 Pentacholorobiphenyl	ND	ND	ND	5 ng g^{-1}
233′4′6 Pentacholorobiphenyl	26.41±0.60	ND	13.10±1.21	5 ng g^{-1}
22′33′46' Hexacholorobiphenyl	ND	ND	ND	5 ng g^{-1}
22′3455' Hexacholorobiphenyl	ND	ND	ND	5 ng g^{-1}
22′34′5′6 Hexacholorobiphenyl	ND	ND	ND	5 ng g^{-1}
22′355′6 Hexacholorobiphenyl	ND	ND	ND	5 ng g^{-1}
22′33′46' Hexacholorobiphenyl	ND	ND	ND	5 ng g^{-1}
233′44′5 Hexacholorobiphenyl	ND	ND	ND	5 ng g^{-1}
233′44′6 Hexacholorobiphenyl	5.80±0.19	ND	7.22±0.68	5 ng g^{-1}
22′33′4′56 Heptacholorobiphenyl	ND	ND	ND	5 ng g^{-1}
22′344′5′6 Heptacholorobipheny	ND	ND	ND	5 ng g^{-1}
22′344′5′6 Heptacholorobipheny	ND	ND	ND	5 ng g^{-1}
22′3344′55Octacholorobiphenyl	ND	ND	ND	5 ng g^{-1}
22′33′455′6' Octacholorobiphenyl	ND	ND	ND	5 ng g^{-1}
22′3444′55′6 Octachlrobiphenyl	ND	ND	ND	5 ng g^{-1}
ΣPCBs	32.22	4.75	70.31	—

* BDL: Below Detection Level, ND: Not detected and MDL: Method Detection Limit.

Lahaye et al. (2006) indicated the presence of mercury (105 μg g^{-1}) and cadmium in the prey fishes including the myctophid fish, *Notoscopelus kroeyeri*. Bargagli (2005) reported the presence of Cd, Cu, Hg and Zn in the internal organs of few myctophids from Kerguelen Islands, Atlantic Ocean and Mediterranean Sea with order of distribution being muscle > kidney > liver.

In conclusion, the possibility of accumulation of persistent organic compounds in myctophids fish species are high due to high lipid contents and biomagnification processes. Though the results of the present study indicated the presence of these chemical contaminants in a few myctophid species available in Indian waters, the levels were found to be well below the prescribed limit.

Table 4. Heavy metals content in myctophids (μg g^{-1} wet weight)

Element	Wavelength (nm)	Slit width (nm)	*D. watasei*	*D. luetkeni*	*M. obtusirostre*	MDL
Zn	213.9	1.0	3.72±0.26	15.91±0.51	13.7±0.26	0.25 ng g^{-1}
Cu	324.8	0.5	BDL	BDL	BDL	2.50 ng g^{-1}
Pb	217.0	1.0	BDL	BDL	BDL	0.25 ng g^{-1}
Cd	228.8	0.5	1.19±0.03	14.43±0.26	8.37±0.27	0.50 ng g^{-1}
Mn	279.5	0.2	1.19±0.03	0.75±0.03	0.75±.04	0.50 ng g^{-1}
Cr	357.9	0.2	BDL	BDL	BDL	1.00 ng g^{-1}
Co	240.7	0.2	BDL	BDL	BDL	0.25 ng g^{-1}
Fe	248.3	0.2	BDL	BDL	BDL	0.75 ng g^{-1}
Li	670.8	1.0	0.26±0.03	2.66±0.08	0.66±0.03	0.25 ng g^{-1}

BDL - Below Detection Level

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