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Organochlorine Pesticides and Polychlorinated Biphenyls in Various Tissues of Waterbirds in Nalabana Bird Sanctuary, Chilika Lake, Orissa, India

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Abstract In order to understand whether organochlorine pesticides and polychlorinated biphenyls (PCBs) are responsible for the mortality of waterbirds in Nalaban bird sanctuary in Chilika Lake, the current investigation was carried out in tissues of 11 individuals comprising 7 species of birds. One or more residues were detected in all the tissues of birds analysed. Concentration of $\sum$HCH, $\sum$DDT, and $\sum$PCBs were ranged from below detectable level (BDL)-811 ng/g, BDL-1,987 ng/g and BDL-1,027 ng/g respectively. PCBs levels were less than the food and drug administration's (FDA) action limits. Although varying levels of residues were detected among tissues, they do not appear to be responsible for the mass mortality of waterfowl. However, the need for additional research is heightened when considering that some of the birds are classified as a globally protected species by the international bodies.

Keywords Organochlorine pesticides · PCBs · Chilika lake · Nalabana bird sanctuary · India

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are ubiquitous contaminants in the global environment due to their persistence and lipophilicity they accumulate in the lipid tissues of organisms (Choi et al. 2001). The most affected species are those that feed on contaminated food, causing population declines through long-term reproductive depression and acute poisoning (Porter 1993). In India, mortality of birds due to pesticide poisoning has been reported at various occasions. Muralidharan (1993) attributed aldrin for the mortality of 18 Sarus Cranes and a few granivorous birds in Keoladeo National Park, Bharatpur. Pain et al. (2004) reported monocrotophos to be the culprit chemical for the death of 15 Sarus Cranes again in Bharatpur. Death of 58 aquatic birds comprising six species of water birds has been reported in Okhla Bird Sanctuary, Uttar Pradesh (Sundar 2006). Furthermore, varying levels of pesticide residues have been reported in many species of birds in India (Dhananjayan 2009; Dhananjayan et al. 2011). However, very a few studies have reported contaminants levels in waterfowl in India.

Organochlorines such as HCH and DDT still account for two-third of the total consumption in the country for agriculture and public health purposes respectively (Kumari et al. 2001). Although the use of organochlorine pesticides and PCBs was restricted in India, there usage was allowed for specific purposes. There are several major wintering areas for resident and migratory birds in India. Among these, the Nalabana Bird Sanctury (NBS) in Chilika Lake, Orissa is a wetland of International importance and designated as a "Ramsar Site" in the year 1981. The Chilika lagoon is one of the prime wintering ground for more than one million migratory birds. The pear-shaped lake is about 64.5 km long; its width varies from 18 to 5 km (CDA 2003). A total of 225 species have been reported, including 9 threatened species (Balachandran et al. 2005). Nalabana Island is situated in the Central Sector of the lake. The area of the island is 15.52 sq. km. During 2006 winter thousands of migratory birds died in the NBS, Chilika Lake, and the reason was not clear. Northern Pintail, Northern Shoveler, Brown-headed Gull, Gargany were notable species among them. During a preliminary visit to Chilika Lake in January 2006, dead

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birds 11 individuals comprising 7 species were collected for contaminant analysis. The objectives of this study were to determine the tissue distributions of environmental contaminants such as PCBs and chlorinated pesticides and also examine the whether these contaminant concentrations could be responsible for the mass mortality of waterfowl in Chilika lake.

Materials and Methods

Dead birds were collected from Chilika Lake (19°28′ and 19°54′ N and 85°05′ and 85°38′, E) during January 2006 (Fig. 1; Table 1). All the samples were transported to the laboratory at Salim Ali Centre for Ornithology and Natural History (SACON), Coimbatore by air in chilled condition without delay. All the birds received were dissected to separate tissues, namely brain, liver and muscle were stored in deep freezer till the time of processing. About 10 g of the tissue was ground with anhydrous sodium sulphate and packed in a thimble, which was desiccated overnight prior to extraction. The sample was extracted with 250 mL of 1:1 ratio of dichloromethane and hexane (Merck) in a soxhlet extractor for 8 h and condensed in a rotary flask evaporator to a specific aliquot (5 mL). Extracts with high fat content were subjected to sulphuric acid digestion. The digested extract was filtered through sodium sulfate and evaporated with rotary flask evaporator to near dryness. The cleaned extracts was fractioned by passing through a column of 12 g of wet Florisil eluting with 100 mL of pesticide grade hexane (first fraction) and then with 150 mL of 20 % dichloromethane in hexane (second fraction). The first fraction contained PCBs and *p*,*p*′-DDE and second fraction contained all organochlorine pesticides. The extracts were concentrated using a rotary evaporator to near dryness and residues were reconstituted in 2 mL of hexane.

The final extracts were analyzed for the following organochlorine pesticides; isomer mixture of hexachlorocyclohexane (∑HCH) consisting α, β, γ and δ-HCH (lindane), DDT metabolites, namely *p*,*p*′-DDT, *p*,*p*′-DDE and *p*,*p*′-DDD and the cyclodiene insecticides, heptachlor epoxide and

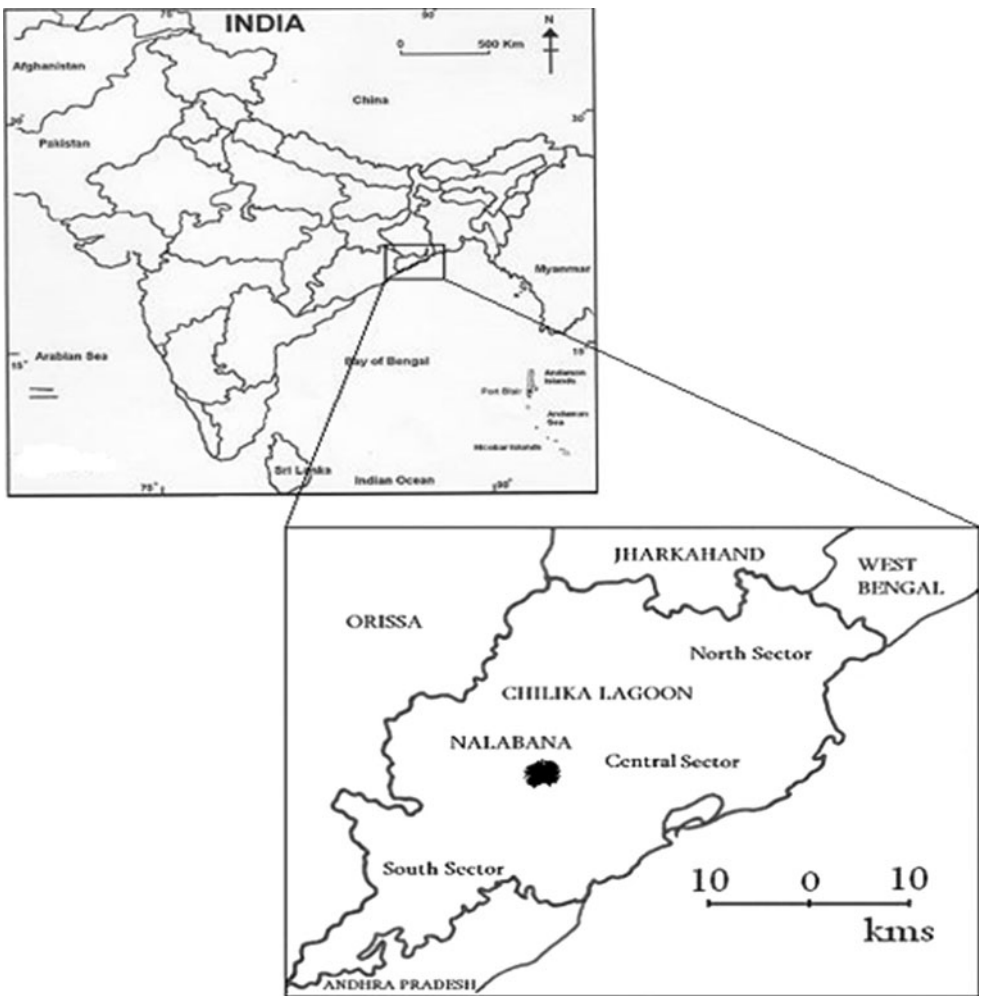

Fig. 1 Map showing the bird collection site at Nalabana Bird Sanctuary, Chilika Lake, India

Table 1 List of bird species collected from Chilika Lake, Orissa, India

Name of the bird	Number of birds	Sex	Date of collection	Major food items[a]
Northern Shoveler *Anas clypeata*	2	M/F	26 January 2006	Largely animal matter, it is not exacting in its food preferences
Northern Pintail *Anus acuta*	2	M	26 January 2006	Largely vegetarian in its food preferences
Garganey *Anas querquedula*	2	M/F	27 January 2006	Largely vegetarian in its food preferences
Lesser Sand Plover *Charadrius alexandrinus*	1	M	26 January 2006	Chiefly small crabs, sand hoppers and marine worms
Brown-headed Gull *Larus brunnicephalus*	2	M/F	27 January 2006	Insects, grubs, slugs and shoots of various crops
Eurasian Spoonbill *Platalea leucorodia*	1	F	26 January 2006	Tadpoles, frogs, mollusks, insects and gettable matter
Ruff *Phiomachus pugnax*	1	F	28 January 2006	Larger quantities of weed seeds

[a] Ali (2002)

dieldrin and 32 congeners of PCBs. An aliquot (1 μL) from the final extract was injected into a Hewlett Packard 5890 series II Gas Chromatography (GC) equipped with a ^{63}Ni electron capture detector and splitless injection port. The GC column employed was DB-608 fused silica capillary column (30 m × 0.32 mm × 0.5 μm thickness; J & W Scientific Inc., Folsom CA) coated with 35 % phenyl methyl polysiloxane. The column oven temperature was programmed from 180°C, held for 3 min, then increased to 270°C at 10°C min^{-1} and held for 20 min. Injector and detector temperatures were set at 250 and 280°C respectively. Nitrogen was used as carrier gas with a column flow rate of 1.5 mL/min. A mixture of organochlorine pesticides (Dr. Ehrenstorfer Laboratories, Germany) and a mixture of 32 PCB congeners (Accustandard, USA) were used as standards. The concentrations of the individual compounds were quantified from the peak area of the sample to that of the corresponding external standard. Recoveries of the compounds from fortified samples (100 ng/mL, n = 3) ranged from 90 % to 102 % and 93 % to 110 % for OCPs and PCBs respectively. Results are not corrected for per cent recovery. Analyses were run in batches of 10 samples plus four Quality Controls (QCs) including one reagent blank, one matrix blank, one QC check sample and one random sample in duplicate. The minimum detection limit for all the compounds analyzed was 1 ng/g. All resides data are expressed as ng/g wet weight.

Results and Discussion

The levels of OCs and PCBs among various tissues of birds are shown in Table 2.

Invariably all the samples detected one or more organochlorine residues. Concentration of total organochlorine pesticides ($\sum$OCPs) ranged between 25 and 1,987 ng/g. The absence of comparable data concerning residue levels in the tissues samples of same species rendered direct analogies, for the majority of toxicants included in this study, extremely difficult. The results reported from surveys on tissues of certain birds are, furthermore, referred to when mention of such information was appropriate as reported by earlier researchers (Dhananjayan et al. 2011).

The maximum level of total HCH was recorded in liver tissues of Northern Shoveler (811 ng/g) followed by liver of Northern Pintail (453 ng/g) and brain of Ruff (182 μg/kg). The concentrations of β- and γ-HCH were the most dominated among isomers of HCHs detected in tissues of birds. HCH isomers were not detected in muscle tissues of Northern Pintail and Lesser Sand Plover and brain of Garganey and Brown-headed Gull. However, the concentration levels of HCH detected in tissues of birds are not believed to affect the survival of avian species. Lindane has not received much attention as far its effects on birds are concerned. Although several studies have been carried out, investigations pertaining to the levels of lindane are a few (Dhananjayan 2009; Dhananjayan et al. 2011). However, varying levels of HCH and its metabolites in tissues of birds included in the present study indicate the regular use of this chemical for agriculture including paddy and pulse (Mukherjee and Gopal 1996). Although varying levels of HCH and its isomers are recorded in many species of birds, their effects on reproductive success are not clear.

The levels of total DDT among tissues ranged between BDL to 1,987 ng/g. Among the metabolites *p*,*p*′-DDT, *p*,*p*′-DDE had significantly higher burden in tissues. The maximum load of *p*,*p*′-DDE was detected in liver tissues of Eurasian Spoonbill (1,954 ng/g) and Ruff (1,351 ng/g). Whereas, *p*,*p*′-DDD and the parent compound *p*,*p*′-DDT were the least detected. In accordance with previous studies (Dhananjayan 2009; Dhananjayan et al. 2011), *p*,*p*′-DDE was the organochlorine pesticide most frequently detected in birds and its concentrations were higher than those of the other compounds. This may be due to its high chemical stability and persistence in the environment. Although the varying concentrations of OCPs detected in the present study, the levels were well below the concentration reported to be responsible for the mortality of birds (Stickel et al. 1970).

Table 2 Organochlorine pesticide residues and PCBs (ng/g, wet wt.) in tissues of birds collected from Chilika Lake, India

Name of the bird	Organ	α-HCH	γ-HCH	β-HCH	δ-HCH	ΣHCH	HE	Dieldrin	*p,p′*-DDD	*p,p′*-DDE	*p,p′*-DDT	ΣDDT	ΣOCPs	ΣPCBs
Northern Shoveler (n = 2)	Liver	ND	103	687	21.4	811	90.3	36.9	ND	107	ND	107	1,857	ND
	Brain	2.63	ND	7.91	ND	10.5	1.01	ND	23.5	35.3	ND	59	81	6.08
	Muscle	7.01	ND	64.2	1.06	72.3	91.1	32.0	7.21	ND	ND	7	275	ND
Northern Pintail (n = 2)	Liver	4.63	247	22.4	179	453	35.2	60.9	ND	181	1.04	182	1,002	ND
	Brain	1.06	13	1.77	9.5	25.3	1.84	1.31	ND	ND	ND	ND	236	87.1
	Muscle	ND	ND	ND	ND	ND	1.09	18.6	ND	24.9	ND	25	45	76.7
Garganey (n = 2)	Liver	16	5.88	9.82	ND	31.7	41.1	32.7	167	148	ND	315	452	30.0
	Brain	ND	ND	ND	ND	ND	ND	ND	39.0	196	9.01	244	244	61.9
	Muscle	ND	ND	2.43	ND	2.43	2.14	ND	4.18	20.2	ND	24	31	10.1
Lesser Sand Plover (n = 1)	Liver	ND	96.7	ND	ND	96.7	ND	70.8	ND	36.6	ND	37	301	80.5
	Brain	3.26	4.44	6.88	6.3	20.9	ND	ND	ND	2.74	ND	3	45	19.2
	Muscle	ND	ND	ND	ND	ND	1.17	ND	ND	50.7	ND	51	52	467
Brown-headed Gull (n = 2)	Liver	ND	ND	1.52	ND	1.52	1.84	1.10	ND	132	1.30	133	139	17.2
	Brain	ND	ND	ND	ND	ND	14.6	5.45	ND	5.11	ND	5	25	14.0
	Muscle	1.15	ND	ND	1.81	2.96	3.79	1.67	1.80	771	ND	773	784	212
Eurasian Spoonbill (n = 1)	Liver	ND	ND	ND	ND	ND	ND	ND	ND	1,954	33.2	1,987	1,987	1,027
	Muscle	2.73	141	9.54	3.32	157	12.6	1.55	ND	808	13.6	822	1,149	209
Ruff (n = 1)	Liver	26.9	24.2	59.4	39.8	150	16.4	ND	ND	1,351	7.51	1,359	1,675	650
	Brain	4.69	97.1	70.8	9.61	182	6.38	2.01	ND	ND	ND	ND	373	63.9
	Muscle	ND	38.1	1.22	5.73	45.1	5.01	2.05	ND	5.98	ND	6	103	ND

HE heptachlor epoxide, Σ-PCBs (total of 32 congeners), *ND* not detected (values were less than detection limit)

The most toxic cyclodiene insecticides such as heptachlor epoxide and dieldrin were ranged from below detectable limit (BDL) to 91.1 ng/g and BDL to 70.8 ng/g respectively. Aldrin, endrin, endrin aldehyde, endosulfan and its isomers were not detected in any of the sample analyzed. Heptachlor epoxide levels recorded in the present study are lower than the LC_{50} values for Japanese Quail, *Coturnix japonica,* 93 ppb and Ring-necked Pheasant, *Phasianus colchinus,* 224 ppb (Heath et al. 1983). The levels were less than 1.5 ppm, which were most definitely associated with decreased reproduction rates in avian experimental study (Henny et al. 1983). The dieldrin levels detected in the current investigation was higher than the concentration reported in liver of yellow-legged herring gull, black-headed gull and woodcock (15, 10 and 23 ng/g respectively) from coastal areas of Campania, Italy (Naso et al. 2003). Further, it may be noted that the Long and Morgan "Effects Range-Low" (ER-L) value, (i.e, the contamination level above which adverse biological effects are occasionally observed), is listed as 0.02 μg/kg for dieldrin (Long et al. 1995). However, the levels of dieldrin are not believed to affect the survival of avian species with this concentration.

Total PCBs concentration ranged from BDL to 1,027 ng/g among various tissues of birds (Table 2). The levels of PCBs were the second most dominant compounds after *p,p′*-DDE, among various organochlorines analysed. The maximum concentration of total PCBs were detected in liver tissues of Eurasian Spoonbill (1,027 ng/g) followed by Ruff (650 ng/g) and Brown-headed Gull (212 ng/g). However, PCBs levels recorded in this study were less than the concentration reported by Naso et al. (2003) in similar food habits of birds like yellow-legged herring gull (3,343 ng/g) and black-headed gull (2,079). These levels were well below food and drug administration's action limit of 2,000 ng/g (Gundersen and Pearson 1992) and also less than the no observable adverse effect concentrations (NOAEC) limit in the US for bald eagle (Wiemeyer et al. 1993).

As far as the total pesticide residue burden is considered in different species, Eurasian Spoonbill had the maximum levels of OCPs followed by Northern Sholveler and Ruff. The lowest pesticide burden was detected in Lesser Sand Plover (Fig. 2). While the maximum total PCBs was detected in Eurasian Spoonbill and Ruff, the minimum was observed in Northern Sholveler (Fig. 3). In general, the liver tissues of birds accumulated higher concentration of organochlorine chemical residues. The organochlorine accumulation load among various tissues of birds are in the order of liver > muscle > brain. Levels OCPs and PCBs measured in tissues of birds reflect the widespread occurrence of these pesticides in the environment. Although, the measured levels in various tissues of birds were lower than those found in field birds that reported poor hatching success, the chemical substances considered in this study are toxic, persistent, and they biomagnify in lipid-rich tissues throughout the foodchain. However, the levels recorded in the tissues of birds in the current investigation do not appear to be responsible for

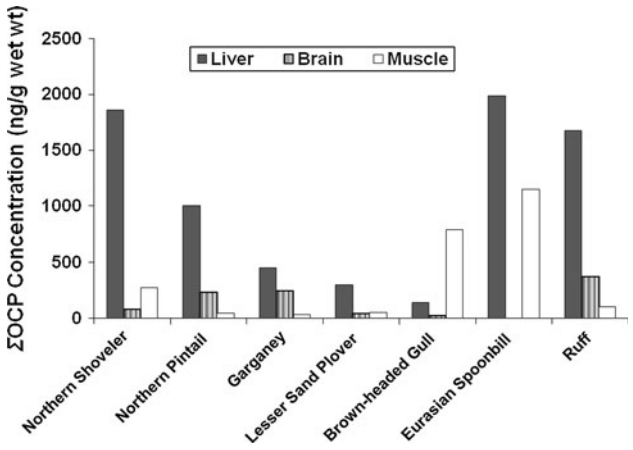

Fig. 2 Variation in concentration of total OCPs among different tissues of birds collected from Chilika Lake, Nalabana Bird Sanctury, Orissa

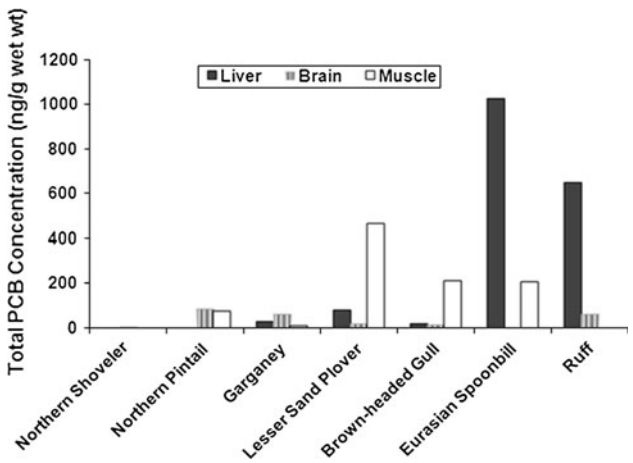

Fig. 3 Variation in concentration of total PCBs among different tissues of birds collected from Chilika Lake, Nalabana Bird Sanctury, Orissa

the mass mortality of waterfowl in the study locations. In order to estimate the potential risk for birds, the threshold levels known and mentioned in the literature must considered carefully, although threshold levels vary among chemicals compounds, bird species, and other environmental factors.

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