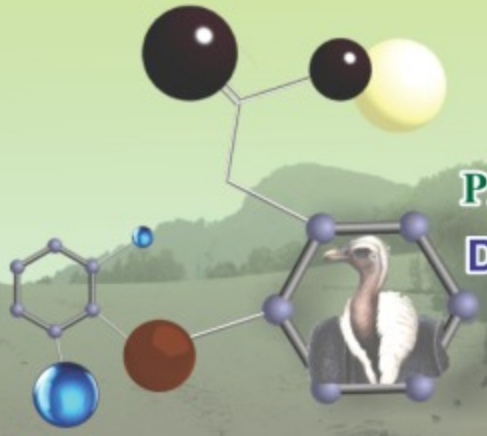
Monitoring and Surveillance of Environmental Contaminants in Birds in India

Final Technical Report

Submitted to

Ministry of Environment, Forest and Climate Change

Government of India



Principal Investigator

Dr. S. Muralidharan

Research Personnel

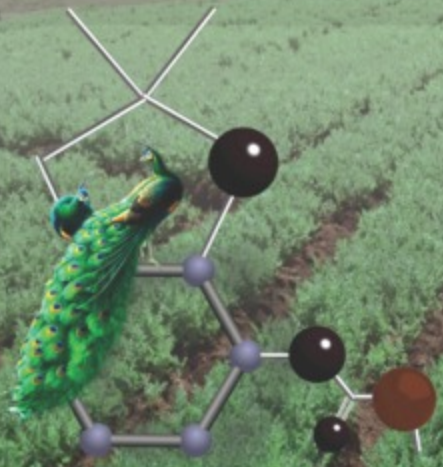
Mr K Nambirajan

Dr K Ganesan

Ms V Kirubhanandhini

Dr S Jayakumar

Dr N Saravana Perumal



Sálim Ali Centre for Ornithology and Natural History

(A Centre of Excellence under the Ministry of Environment, Forest & Climate Change, Govt. of India)

Coimbatore – 641 108, Tamil Nadu.

June 2017

Photos : Aditya Roy

Final Technical Report

Published by : **Sálim Ali Centre for Ornithology and Natural History**

Anaikatty, P.O., Coimbatore 641 108. Tamil Nadu

Tel : 91 422- 2203123, 2203101

Email : salimali@sacon.in URL : www.sacon.in

Suggested Citation : Muralidharan S., Nambirajan K., Ganesan K., Kirubhanandhini V., Jayakumar S., and Saravana Perumal N. (2017). Monitoring and surveillance of environmental contaminants in birds in India. Sálim Ali Centre for Ornithology and Natural History. Final Technical Report 184. pp 203.

For correspondence : Dr S Muralidharan, muralidharan.s@sacon.in, ecot_mur@gmail.com

Front cover : Agro-ecosystem : Designed by Mr S Suresh Marimuthu

Front cover inside : Red-crested Pochard, House Crow, Barn Owl & Himalayan Griffon

Back cover : Glossy Ibies & Cattle Egret in agro-ecosystem

Design & Layout : Mr K Nambirajan

Monitoring and Surveillance of Environmental Contaminants in Birds in India

Final Technical Report

Submitted to

Ministry of Environment, Forest and Climate Change,
Govt. of India

Principal Investigator : Dr S Muralidharan

Research Personnel : Mr K Nambirajan
Dr K Ganesan
Ms V Kirubhanandhini
Dr S Jayakumar
Dr N Saravana Perumal

Sálim Ali Centre for Ornithology and Natural History
(A Centre of Excellence under the Ministry of Environment, Forest & Climate Change, Govt. of India)
Coimbatore – 641 108, Tamil Nadu.

June 2017

Part I
Proforma for Final Technical Report

1	Project title	:	**Monitoring and Surveillance of Environmental Contaminants in Birds in India**
2	Principal investigator	:	**Dr S Muralidharan** Senior Principal Scientist, Division of Ecotoxicology, Sálim Ali Centre for Ornithology and Natural History, Anaikatty, Coimbatore 641 108, Tamil Nadu. Email Id : ecot_mur@yahoo.com, muralidharan.s@sacon.in Phone : +91 422-2203123, 91 9443101589
3	Research personnel	:	Mr K Nambirajan Dr K Ganesan Ms V Kirubhananthini Dr S Jayakumar Dr N Saravanaperumal
4	Funding agency	:	Ministry of Environment, Forest and Climate Change, Govt. of India.
5	Number and date of Sanction letter	:	15-10/2009/WL-I
6	Duration of the project	:	2010-2013, extended till February 2017
7	Total outlay of the project	:	Rs. 48,36,375/-
8	Date of start of project	:	April, 2010
9	Date of completion of project	:	February, 2017

Part II Detailed project report

Part III

Executive summary

Persistent organic pollutants (POPs) are toxic chemicals that are resistant to degradation in the environment and biota. Due to their fat soluble nature and resistance to degradation, ingestion of certain classes of POPs by animals leads to bioaccumulation throughout their lives, generally in the fatty tissues, and biomagnification through food chain. Among the POPs, organochlorine pesticides (OCPs) are highly prevalent in vertebrates. Enormous quantities of such man-made chemicals are being used in modern agriculture to increase productivity to meet the ever increasing food demand. Despite obvious benefits, their indiscriminate use has resulted in long-term environmental and ecological impacts.

Birds are one of the major victims of environmental contaminants as they occupy a wide range of trophic levels in different food chains. Carson (1962) was the first to draw attention to the sensitive nature of birds to the deleterious effects of pesticides. She made the world know about this through her book “Silent Spring”. Birds have been used widely as bioindicators for environmental pollution, particularly persistent organochlorines (OCs), which are considered to be potential endocrine-disturbing chemicals in wildlife. The ill effects of pesticides on wildlife, particularly birds have been an ongoing concern.

Despite proliferation of different types of new-generation pesticides, OCs are sure to be of concern for many more years to come. Based on the information available in India, the impacts of pesticides on birds, including many threatened species are clear. While there is proof beyond doubt for a few species of birds, there are indications of toxic effects in other cases. In India, unfortunately, little systematic work is done till-date to monitor the pesticide residue levels in various biological components, and to evaluate their ill effects.

Towards understanding the impact of contaminants on ecosystem components using bird as an indicator, a study was carried out with the following objectives; 1. Monitor residue levels of persistent chemicals in birds and generate a database, 2. Identify chemicals responsible for mass mortality of birds across the country, and 3. Assess the effectiveness of guidelines on usage of major chemical pesticides in the country. We measured the levels of heavy metals, diclofenac and esterase activities in addition to pesticides in several species of birds. The findings are organized and presented chapter wise.

Efforts were made to collect dead birds from all over India. Opportunistic sampling strategy and organized field visits were followed to collect samples of dead birds. Post-mortem examinations were conducted either in the field or at SACON laboratory. Tissues such as muscle, liver, kidney, gut content, feathers and bones were collected, wrapped in aluminum foil and labeled. All these tissues were brought to SACON over ice, and preserved at -20 $^{\circ}$C until chemical analyses. Multi-residue extraction procedure adopted by Anastassiades *et al.,* 2003 was followed to extract pesticide residues from bird tissues. Residues were analysed with Hewlett Packard 5890 Series II Gas Chromatograph equipped with Ni^{63} Electron Capture Detector. GC-MS/MS was used for confirming chemicals involved in poisoning cases, wherever required. Results are expressed as ng/g ±SE (wet weight).

During the study period (2010 and 2014), 777 birds belonging to 90 species were received dead from nine states of India, namely Assam, Gujarat, Jharkhand, Kerala, Karnataka, Maharashtra, Tamil Nadu, Uttar Pradesh and Uttarkhand. Notable species were Eurasian Griffon, Indian

White-backed Vulture, Slender-billed Vulture, Black-crowned Night Heron, Barn Owl, Eurasian Eagle Owl, Emerald Dove, Spot-billed Pelican, Painted Stork, White Ibis, Sand Piper, White-checked Barbet, Shikra, Eurasian Golden Oriole, Red-crested Pochard, Black Winged Stilt and Common Moorhen. While incidences of poisoning among birds have been dealt separately, data on pesticide residues HCH, DDT, endosulfan, heptachlor, alachlor, dieldrin, dicofol and chlorpyrifos, metabolites and isomers wherever applicable have been compiled to check the overall load, variation among species, tissues and between sexes. Spatio-temporal variations over the years and variations based on feeding habits have also been looked into to understand the implications with reference to usage patterns of the pesticides or policies of the government that are in existence.

Incidences of poisoning: Based on circumstantial evidences, poisoning was suspected in the mortality of many species of birds. Subsequent laboratory analyses confirmed poisoning in seven incidences involving thirteen species of birds. Around 50 Peafowl died on the 7th August 2012 in Anaikaradu Forest range near Keveriammapatti, Dindigul District, Tamil Nadu. After the investigation and based on circumstantial evidences, it was confirmed that the mortality was due to unintentional poisoning with phorate and carbofuran.

In April 2010, 33 fish-eating birds comprising six species, namely Spot-billed Pelican *Pelecanus phillippensis*, Little Egret *Egretta garzetta*, Painted Stork *Mycteria leucocephala*, Eurasian Spoon-bill *Platalea leucorodia*, Oriental White Ibis *Threskiornis melanocephalus* and Black-crowned Night-Heron *Nycticorax nycticorax* died in Arignar Anna Zoological Park, Chennai within three hours of feeding. Clinical signs such as dullness, drooping wings, regurgitation and diarrhea were observed. Physical examination of dead birds revealed that they were otherwise healthy. Histopathological examinations of the gizzard, liver, kidney and intestine revealed mild to diffused congestion and hemorrhage. The ingested fish recovered from gizzard of dead birds had high concentrations (up to 30 ng/g) of phosphamidon, an organophosphate pesticide, and was confirmed to be the cause for mass mortality.

Forty Demoiselle Cranes were reported dead in and around Amreli district, Gujarat during December 2012. Field survey was conducted in areas where the Cranes dead under suspicious circumstances and gathered circumstantial evidences. Laboratory investigations on dead Cranes confirmed the mortality to be due to phorate poisoning. On 23nd December 2012, another Demoiselle Crane that was spotted sick the previous evening collapsed in front of us at Sakria lake and we conducted post-mortem of the bird in the field and transported samples to the laboratory at SACON for contaminant analysis. Excessive salivation, foam around mouth, breathing difficulties, teary eyes, inability to fly, tottered walk and bleeding in the head and beak was noticed. These symptoms confirmed that a rodenticide was responsible for the death.

Deliberate poisoning of birds was reported in a village called Thiruthangal, near Sivakasi in Viruthunagar Dt, Tamil Nadu during July 2013. It was learnt that nomads in the area poison fish-eating birds using pesticide loaded fish as baits. Further, it was understood that the nomads slit up fishes, place granules of carbofuran, close and strategically place these baits on the banks of village ponds.

Between November 2011 and February 2012, several crows died in and around Jamshedpur, and the number of death was estimated at 2000. There were also reports on the death of

crows in other parts of Jharkhand, Bihar, West Bengal and Odisha. One of the dead crows we received from Jamshedpur had residues of methyl parathion and HCH. While the level of HCH isomer was not indicative of toxicity, methyl parathion could indicate high exposure. This could be treated as an evidence of poisoning. However, due to inadequate sample size, we could not conclude that all the crows might have died due to methyl parathion poisoning. Besides, there were also unconfirmed reports on the role of H_5N_1 virus in the death of crows in Jamshedpur and Khurda district in Odisha.

Another case of poisoning was in House Swift *Apus affinis* in Vandiyur Mariamman temple pond in Madurai district, Tamil Nadu during April 2012. Laboratory analyses confirmed that the mortality of around 150 Swift was due to DDT. It may be noted that DDT was banned for agriculture and permitted to be used only for controlling malaria. It is a clean case of illegal use of DDT.

Samples of birds, namely Red-crested Pochard and Common Moorhen received from Sitarganj Forest Range, Uttarakhand had high levels of chlorpyrifos, suggestive of poisoning. We have also confirmed reports on the death of Large Cormorant *Phalacrocorax carbo* and Greater Pelican *Pelecanus onocrotalus* (around 50) in Kaziranga National Park, Assam, Indian Peafowl *Pavo cristatus* in Rajasthan (12 Nos), Madhya Pradesh (7 Nos), Maharashtra (13 Nos) and Tamil Nadu (33 Nos), Blue Rock Pigeon *Columba livia* in Pune (38 Nos) and Ahmedabad (>50 Nos), Cattle Egret *Bubulcus ibis* in Amreli District of Gujarat and crows in Vellalore dumping yard at Coimbatore during the study period. While circumstantial evidences were pointing towards chemical poisoning, samples of tissues could not be obtained for residue analysis.

Residues of organochlorine pesticides: Organochlorine pesticide residues were determined in 90 species of birds collected between 2010 and 2014. Among the various OCs analysed, isomers of HCH (41%) and metabolites of DDT (29%) contributed maximum to the ∑-OCPs followed by ∑-endosulfan (12%), dieldrin (8%), ∑-heptachlor (6%), dicofol (3%) and alachlor (1%). Among the isomers of HCH, β (beta)-HCH accounted for 47% of total HCH, while γ (gamma)-, α (alpha)- and δ (delta)-HCH accounted for 27%, 22% and 4% respectively. Of all the metabolites of DDT, *p,p'*-DDE contributed the maximum (63%) to the ∑-DDT residues, indicating its highest persistence in bird tissues.

Maximum level of total OCs were recorded in liver tissues of Himalayan Griffon *Gyps himalayensis* (2600 ± 564 ng/g) received from Sivasagar district in Assam and minimum in muscle tissues of Rose-ringed Parakeet *Psittacula krameri* (13 ± 4 ng/g) collected from Shree Gadhada Mahajan Panjarapole, Bhavnagar, Gujarat. Species, namely Little Cormorant *Phalacrocorax niger*, Besra Sparrow-hawk *Accipiter virgatus* and Indian White-backed Vulture *Gyps bengalensis* recorded 2356.4, 2011.5, and 1422.5 ng/g of total OCs respectively.

Significant variations in HCH residues were observed among the various species of birds studied (ANOVA, $P < 0.05$). Comparatively higher load of total HCH (> 2000 ng/g) was recorded in House Swift *Apus affinis*, Spotted Owlet *Athene brama* and Black Kite *Milvus migrans*. Maximum concentration of ∑-DDT was recorded in Barn Owl *Tyto alba* (4881.4 ng/g) collected from Ahmedabad, Gujarat. Interestingly the same species received from the agrarian belt of Mayiladuthurai, Tamil Nadu also had high concentration of DDT residues (2248.2 ng/g). Other notable species those recorded higher residues of DDT were Little Egret *Egretta garzetta*

(2822.5 ng/g), Median Egret *Mesophoyx intermedia* (2113.09 ng/g), Black Kite *Milvus migrans* (1988.2 ng/g) and Spotted Owlet *Athene brama* (829.9 ng/g).

Of all the tissues studied, the maximum accumulation of OC residues was in liver followed by kidney, and minimum in muscle. Although, the differences in accumulation were not significant among tissues (ANOVA, P>0.05), significant differences were observed in OC levels among the various species of birds studied. ∑-OC pesticide load in the birds studied, based on their feeding guilds, was in the following order: insectivore> carnivore> piscivore> omnivore> granivore. Resides of all organochlorine pesticide tested showed higher load of accumulation in birds of Gujarat followed by Assam, Tamil Nadu and Kerala. It is to be noted that the number of birds received dead from Uttar Pradesh, Jharkhand, Uttarakhand, Karnataka and Maharashtra was not many.

Organochlorine pesticide residues were detected in appreciable concentrations in many species of birds studied. Although the residue levels recorded in the current study are not indicative of toxicity except House Swift, these residues can be expected to harm birds at higher concentration, if exposure continues. Although we have phased out many OCs due to their established ill effects on ecosystem components in India in the recent past, whatever we used till recently are unfortunately expected to be in the environment for many more years. As the OC pesticides have adverse effects not only on birds and aquatic life forms, but also on man, regular monitoring of their residue levels in select environmental components is strongly advised.

Residues of diclofenac in the tissues of vultures: Vultures play an important role in the ecosystem by scavenging on dead animals, preventing the spread of diseases and keeping environment clean. They are obligate scavengers with a very low probability of acting as source of infection. The Indian White-backed Vulture *Gyps bengalensis* was once one of the most common raptors in the Indian subcontinent. Populations of three resident *Gyps* species, namely the Indian White-backed Vulture *Gyps bengalensis*, Indian Vulture *Gyps indicus* and Slender-Billed Vulture *Gyps tenuirostris*; endemic to South Asia have decreased by more than 90% since the mid- 1990s, and is continuing to decline. They are at high risk of global extinction, listed as Critically Endangered (IUCN 2004) and categorized under Schedule I of Indian Wildlife Protection Act 1972; 2002 (Amended).

Experimental studies showed that the cause of mortality of vultures to be renal failure caused by diclofenac, a non-steroidal anti-inflammatory drug. The drug was used by veterinarians to treat domestic livestock extensively in the 1990s. Residues of the drug were also found in carcasses of domesticated ungulates available for vultures to feed in India. It was evident that those vultures scavenging on cattle carcasses treated with diclofenac shortly before death, died due to poisoning. Based on these studies, Government of India, Pakistan and Nepal banned the veterinary usage of diclofenac in 2006 to prevent further decline in the vulture population. However, subsequent studies showed a very high prevalence of diclofenac in the post ban period that continued to cause a decline of vulture population in Indian sub-continent.

Although, the present study documented mainly the impact of pesticides and heavy metals on birds, since diclofenac has been proved to be responsible for population decline in vultures, we

also monitored the residue levels in them. Method adopted by Oaks *et al.* (2004) was followed with slight modification for sample processing and analysis. Diclofenac was analysed with High-Performance Liquid Chromatography (Agilent 1100 Series) equipped with Diode Array Detector, fitted with Agilent Zorbax SB-C18 (4.6 mm x 150 mm, 5 µm) column.

Between 2011 and 2014, totally 44 dead vultures comprising two species, namely Indian White-backed Vulture *Gyps bengalensis* (32) and Himalayan Griffon *Gyps himalayensis* (12) were collected for this study from three states, namely Gujarat, Assam and Tamil Nadu on opportunistic basis.

Of the 32 dead White-backed Vultures analysed, 68.75% of them had detectable levels of diclofenac residues ranging from 62.28 ng/g in kidney to 272.20 ng/g in liver. Pattern of diclofenac detection among the materials tested was in the following order; kidney (50.0%) > liver (43.75%) > gut content (25.0%). Of the 30 kidney samples analysed, 50.0% of them had detectable levels of diclofenac residues which ranged from 62.28 ng/g to 266.52 ng/g with a mean value of 133.07 ng/g. Of the 32 liver samples analysed, 43.75% of them had detectable levels of diclofenac residues ranging from 87.94 ng/g to 272.20 ng/g with a mean value of 153.69 ng/g. Twelve samples of gut content were analysed, and of which 25% of samples had detectable levels of diclofenac residues ranging from 70.90 ng/g to 149.03 ng/g with an average of 111.15 ng/g. Between the organs, liver had higher level of diclofenac residues (153.69 ng/g) than kidney (133.07 ng/g).

Out of twelve Himalayan Griffon vultures analysed for diclofenac residues, 75% of them had detectable levels. The frequency of diclofenac detection among the organs/gut content of Himalayan Griffon was in the following order; liver (66.67%) > kidney (55.56%) > gut content (37.50%). Nine kidney samples were analysed for diclofenac residues, and of which 55.56% of them had detectable levels of diclofenac residues ranging from 139.69 ng/g to 325.66 ng/g with a mean value of 209.56 ng/g. Among the twelve liver samples analysed, 66.67% of them had detectable levels of residues, and it ranged from 153.51 ng/g to 355.17 ng/g with a mean value of 256.12 ng/g. Eight samples of gut content were analysed, and of which 37.5% of samples had detectable levels of diclofenac residues with an average of 261.62 ng/g. While the minimum was 166.32 ng/g, maximum was 411.73 ng/g. Accumulation of diclofenac residues among the organs/gut content of Himalayan Griffons was in the following order; gut content (411.73 ng/g) > liver (256.12 ng/g) > kidney (209.56 ng/g). Himalayan Griffon *Gyps himalayensis* had higher prevalence of diclofenac residues than the Indian White-backed Vulture *Gyps bengalensis.*

This study revealed that diclofenac still continues to kill vultures even long after its ban for veterinary use in India (2006). The prevalence of diclofenac in vulture carcasses reflected only a small and insignificant drop in the usage of the drug after its ban in India.

Heavy metal contamination: Heavy metals are considered as critical contaminants in the environment, as they are ubiquitous, highly persistent non-biodegradable, and accumulate in food chains. However, large number of metals in trace quantities is also essential for biological sustenance. Virtually even essential metals can turn toxic to all organisms including birds and humans, if exposure levels are high. Birds are one of the major victims of metal contamination as they occupy a wide range of tropic levels. Over the years among wildlife, birds have served as bioindicators for a number of environmental contaminants, especially heavy metals as they

are visible, widely distributed in the ecosystem, sensitive to toxins, high on the food chain and ecologically versatile, and also as they live in diverse habitats.

Differences in metal accumulation can be related to different foraging habitats. Diet pattern and types, body condition and age could also influence metal levels in birds. Similarly, time of egg laying, species metabolic difference and enzymatic detoxification mechanism can cause differences in metal concentration. Studies show that even closely related bird species may differ in metal accumulation and excretion. The age of birds is also an important factor for metal accumulation. Some elements are known to accumulate over time, resulting in increasing accumulation with age, although growing nestlings may also accumulate higher concentrations than adults.

In India, studies on metal contamination on birds are a few. There is a growing concern to examine metal contamination in birds and interpret their levels; thereby appropriate measures may be taken to conserve the species. The present study was conducted with an objective to document the levels of accumulation of metals such as Cu, Pb and Cd contamination in different species of birds in select states in India.

The dead birds were collected from various parts of the country on opportunistic basis as explained elsewhere. Upon autopsy, select organs such as liver, kidney and muscle were dissected out to study the metal levels. Tissues were digested using specific mineral acids such as nitric, perchloric and hydrogen peroxide using Microwave Digestion System. Digested solutions were analysed using Atomic Absorption Spectrometer for metals such as Cu, Pb and Cd. Appropriate quality control measures were followed during the analysis.

Data on 139 individuals comprising 18 species of birds are presented in detail.

Population of Peafowl is believed to be stable in the country in the absence of any evidence for any substantial decline. Concentration of metals was estimated in tissues, namely liver, kidney and muscle. Cd accumulated the highest in liver (1.24 ± 0.19 µg/g) and lowest in muscle (0.77 ± 0.41 µg/g). Pb accumulated the maximum in kidney (1.31 ± 0.39 µg/g) and minimum in muscle (0.99 ± 0.06 µg/g). There was significant difference in the magnitude of contaminations among organs.

Bar-headed Goose had detectable concentrations of all the metals analyzed. Kidney had higher accumulation of all metals than liver, except Cu which was high in both liver (7.36 ± 0.95 µg/g) and kidney (5.67 ± 1.82 µg/g). Muscle, liver and kidney were the tissues analyzed in Painted Stork. Copper was higher in concentration than other two metals. Concentrations of all three metals were the highest in liver; Cu -5.6 ± 1.34 µg/g, Pb - 0.89± 0.26 µg/g and Cd - 2.25 ± 1.01 µg/g. The level of accumulation was significantly different between muscle and liver.

In Indian Black Ibis, Cu levels ranged between 0.18 µg/g and 1.08 µg/g in kidney. Liver had the maximum level of Cu (2.59 ± 0.68 µg/g). Significant variations were observed in Cu levels between liver and kidney. Near equal levels of Cd was observed in liver and kidney, and the liver to kidney ratio was 1. This indicates a balance in accumulation and regulation of overall body burden of Cd.

Among the nine individuals of Indian Pond Heron collected from Gujarat (n=5) and Kerala (n=4) between 2011 and 2012, kidney recorded the maximum level of all three metals; copper: 2.11

± 0.25 µg/g, cadmium: 0.23 ± 0.04 µg/g and lead: 1.44 ± 0.34 µg/g. All metals except Cd, had significantly (P<0.05) different concentration among organs studied. Levels observed in the present investigation are comparatively lower than the levels recorded in earlier studies. Further it may be noted that levels of Pb in the range of 3 µg/g to 6 µg/g was reported to be toxic to birds. Since levels recorded in the present study are lower than any known toxic level, it can be said that at present this species is not under any major threat.

Between 2010 and 2012, 24 individuals of Cattle Egret were collected dead from Tamil Nadu (n=4), Kerala (n=10) and Gujarat (n=10). Average Pb level was found to be higher in kidney (0.92±0.44 µg/g) than liver (0.74±0.52 µg/g). Mean Cd level was found to be more or less the same in both the tissues. Liver had significantly higher levels of Cu (3.08 ± 0.47 µg/g) than kidney (1.86 ± 0.32 µg/g;). These levels could be treated as reference values as there are no other background concentrations available to define toxicity of metals in Indian avifauna.

Black Kite is one of the major victims of kite flying every year during January in Gujarat, particularly Ahmedebad. Totally 26 individuals were examined for metal contamination. Between the two organs, while kidney had the highest accumulation of Pb (1.20±0.76 µg/g) liver had the maximum accumulation of Cu (3.32±2.28 µg/g) and Cd (0.84±0.40 µg/g). Variation in accumulation of Cu between liver and kidney was found to be significant.

In the current study, three species of vultures were examined for metals. A total of 22 individuals comprising Eurasian Griffon Vulture (LC), Himalayan Griffon Vulture (NT) and White-backed Vulture (CE) were collected from Tamil Nadu, Gujarat and Assam. Of all three species of vultures, Indian White-backed Vulture had the maximum concentration of copper in liver (6.19 ± 0.49 µg/g) and Eurasian Griffon the minimum (liver 2.21 ± 0.66 µg/g). Lead (1.70 ± 0.15 µg/g) and cadmium (1.93±0.71) accumulated the maximum in the liver of Himalayan Griffon Vulture. Levels of lead above 2.0 and 8.0 µg/g ww in livers and above 2.0 and 6.0 µg/g ww in kidneys in wild birds (particularly raptors) cause ‘‘toxic’’ and ‘‘fatal’’ effects respectively. Based on these guidelines, lead observed in the present study in liver and kidney are not toxic.

In Demoiselle Crane, kidney showed highest accumulation of copper (4.11 ± 0.79 µg/g) followed by lead (1.83 ± 1.13 µg/g) and cadmium (0.30 ± 0.09 µg/g). Copper (0.23 µg/g), and lead (0.16 µg/g) were observed to be the least in concentration in muscle while cadmium was the least in liver (0.15 µg/g) tissues. The pattern was the same in Sarus Crane as well. In Red-wattled Lapwings, cadmium (2.13 ± 0.72 µg/g), copper (1.89 ± 0.52µg/g) and also lead (1.67 ± 0.55 µg/g) accumulated the maximum in kidney. Least concentration of all the three metals was in muscle tissues. Interestingly, concentrations of all the three metals were more or less the same in all the liver tissues examined.

Of the three metals investigated, in Ring-necked Dove. muscle had the maximum of copper 5.19 ± 0.64 µg/g and liver the minimum (2.39 ± 1.16 µg/g). Cadmium was the highest in kidney (0.34 ± 0.12µg/g) and lowest in muscle (0.11 ± 0.04 µg/g). Lead accumulated the maximum in kidney (1.23 ± 0.21 µg/g) and the minimum in muscle (0.28 ± 0.07 µg/g). In Rose-ringed Parakeet, lead (1.30 ± 0.22 µg/g) and cadmium (0.84 ± 0.26 µg/g) concentrations were the maximum in kidney while cadmium was the least in muscle (0.14 ± 0.07µg/g).

In Barn Owl, levels of Cu (6.5 µg/g) and Pb (0.67 µg/g) were the maximum in liver. Cd (1.45 µg/g) was the maximum in kidney. Muscle accumulated the least of Cu (3.79 ± 1.8 µg/g), Pb (0.024 ± 0.021 µg/g) and Cd (0.621 ± 0.084 µg/g). Five crows collected from two states, namely

Tamil Nadu (3) and Gujarat (2) during 2011 and 2012 showed detectable levels of metals. Muscle tissue had the maximum level of Cu (4.00 ± 0.66 µg/g) and kidney the minimum (2.40 ± 0.72 µg/g). Level of Pb was lesser than Cu and Cd, and muscle had the least (0.37 ± 0.04 µg/g). Cd accumulation was the highest in kidney (1.15 ± 0.35 µg/g) and lowest in muscle (0.38 ± 0.10 µg/g).

In the present study to understand the variation in metal contamination with respect to feeding guilds, all the birds studied were grouped under six feeding guilds, namely granivore, frugivore, insectivore, piscivore, carnivore and omnivore. As feeding guilds differentiate birds by diet, they may also influence risk of exposure to contaminants. Concentration of each metal under the feeding guild was worked out without paying importance to species or organs to understand feeding guild-based variation in the magnitude of contamination.

Accumulation of copper was the maximum in piscivorous (4.62 ± 0.85 µg/g) birds followed by insectivorous birds (2.53 ± 0.23 µg/g), and minimum in granivorous (0.94 ± 0.16 µg/g) birds. Variation among feeding guilds was significant. Insectivorous birds which feed on primary consumers (small body size), accumulated lower levels of metals than other feeding guilds. Lead was the highest in piscivorous birds (1.94 ± 0.64 µg/g) followed by granivorous birds (1.73 ± 0.26 µg/g). The lowest concentration of lead was found in omnivorous species (0.79 ± 0.06 µg/g).

Carnivorous species of birds had the maximum level of cadmium (1.20 ± 0.10 µg/g) followed by piscivorous species (0.93 ± 0.20 µg/g), while granivorous species (0.41 ± 0.08 µg/g) had the minimum (0.54 ± 0.06 µg/g). Although it might look obvious, the relatively higher level of Cd in carnivores and piscivorous birds may be due to the polluted habitat in which they live and forage. With respect to variation in contamination among feeding guilds, piscivorous and carnivorous birds had accumulated the maximum level of heavy metals than others. Frugivorous and insectivorous had relatively less accumulation. This clearly showed that species at higher tropical level accumulated the maximum through food chain.

Among all the states, maximum number of the individuals of birds was collected from Gujarat. Gujarat being an industrialized state, rivers and other aquatic systems are highly polluted. Of all the species studied four species of birds, namely Painted Stork (NT), Indian White-backed Vulture (CE) and Himalayan Griffon Vulture (NT) need special care as their conservation status would demand.

It is obvious that the effects of heavy metals on birds will vary based on several parameters. In India many species of birds listed above are not clearly studied even from ecological point of view. Although, none of the metals studied was high enough to indicate any serious threat, detailed study on the habitat, three and behavior of each and every species of birds is needed in addition to studying contaminants to understand their ill effects.

Plasma and brain cholinesterase activities: Frequent poisoning of wild birds in India as a result of exposure to anticholinergic pesticides each year necessitates rapid and reliable diagnostic techniques to determine the cause of death. Currently, two main diagnostic protocols are routinely employed in our laboratory to detect most commonly used pesticides in birds, namely a multi-residue screening and whole brain/plasma cholinesterase activity measurement. A diagnosis based on depressed whole plasma/ brain cholinesterase activity

requires the knowledge of normal plasma/ brain cholinesterase activity values of a wide range of bird species. Hence, the purpose of the study was to establish a reference range of normal whole brain and plasma cholinesterase activities, which will enable Ecotoxicologists to evaluate suspected cases of organophosphorus and carbamate pesticide exposure in wild birds in India.

In this context, plasma/ brain acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and carboxylesterase (CbE) activities were characterized in 28 species of wild birds those died due to kite injuries in and around Ahmadabad, India between 2011 and 2014.

Cholinesterase activities were measured following the method of Ellman *et al*. (1961), and adapted by Hill (1988). Carboxylesterase was assayed as per the method adopted by Clement and Ehrhadt (1990), and modified by Pope and Karanth (2000). UV-Vis spectrophotometer (PerkinElmer Lambda 35) was used for sample analyses. While 123 plasma samples from 16 species of birds collected during 2011-2014 were examined for AChE and BChE activities, 73 plasma samples collected from 13 species of birds during 2012-2014 were analysed for CbE activity.

Plasma AChE, BChE and CbE activity: Plasma acetylcholinesterase (AChE) activity was significantly different among 16 species of birds examined (ANOVA, $P < 0.05$). It ranged between 0.10 µmoles/min/mL in the Sarus Crane *Antigone antigone* and 4.15 µmoles/min/mL in the Common Moorhen *Gallinula chloropus*. The mean plasma AChE activity was the highest in Blue Rock Pigeon *Columba livia* (2.82 ± 0.77 µmoles/min/mL) while lowest was measured in Indian White-backed Vulture *Gyps benghalensis* (0.97±0.43 µmoles/min/mL).

Plasma butyrylcholinesterase (BChE) activity ranged between 0.29±0.15 µmoles/min/mL in Blue Rock Pigeon *Columba livia* and 1.64±0.51 µmoles/min/mL in Demoiselle Crane *Anthropoides virgo*. Plasma BChE activity also significantly varied among species (ANOVA, $P < 0.05$). Plasma (CbE) carboxylesterase activity was very low compared to that of the other cholinesterase enzymes although they cannot be compared. The highest value recorded was 1.04±0.87 µmoles/min/mL in Indian Peafowl *Pavo cristatus,* while the least was in Indian White-backed Vulture *Gyps benghalensis* 0.07±0.01 µmoles/min/mL.

Brain AChE, BChE and CbE activity: Totally seventy four brain samples comprising 18 species of birds were examined for brain AChE, BChE and CbE activities. Brain acetylcholinesterase (AChE) activity varied significantly among the species (ANOVA, $p< 0.05$). Highest levels of mean brain AChE activity was observed in Black Kite *Milvus migrans* (1.13±0.45 µmoles/min/g), while lowest activity was in Blue Rock Pigeon *Columba livia* (0.29±0.07 µmoles/min/g). Sex-dependent variation in brain cholinesterase activities was examined only in species with three and more individuals in each sex.

Variation in brain butyrylcholinesterase (BChE) activity among the species was highly significant (ANOVA, $p< 0.05$) similar to AChE. Among the species studied, the highest level of mean brain BChE activity was observed in Black Kite *Milvus migrans* (1.42±0.76 µmoles/min/g), whereas lowest level of activity was in Blue Rock Pigeon *Columba livia* (0.41±0.15 µmoles/min/g).

Brain carboxyl esterase (CbE) activity ranged between 0.26±0.10 µmoles/min/g in Black Kite *Milvus migrans* and 0.72±0.10 µmoles/min/g in Median Egret *Ardea intermedia*. The variability

in plasma ChE activity among different species of birds underscores the need for characterizing AChE, BChE and CbE before using the enzymes in biomonitoring studies. Baseline measurements of ChE activity in birds have been virtually absent till date in India apart from the information we have generated in SACON laboratory. The results presented here are expected to serve as a starting point for the use of plasma/ brain ChE activity as a biomarker in Indian birds, aiding field investigations and monitoring risk of exposure of non-target wildlife particularly birds to ChE-inhibiting new generation compounds. It is believed that the reference values presented in this study will enable ecotoxicologists in India to interpret results with greater confidence and reliability.