BIOLOGICAL AND CHEMICAL STUDIES ON AVIAN EXCRETA

Dissertation

**Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of**

**DOCTOR OF PHILOSOPHY
in
ZOOLOGY
(Minor Subject: Biochemistry)**

By

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(L-2008-BS-43-D)**

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CERTIFICATE I

This is to certify that the dissertation entitled, **"BIOLOGICAL AND CHEMICAL STUDIES ON AVIAN EXCRETA"** submitted for the degree of Ph.D., in the subject of **Zoology** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Navdeep Kaur (L-2008-BS-43-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

Major Advisor
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CERTIFICATE II

This is to certify that the dissertation entitled, "**BIOLOGICAL AND CHEMICAL STUDIES ON AVIAN EXCRETA**" submitted by **Navdeep Kaur** (**L-2008-BS-43-D**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of Ph.D, in the subject of **Zoology** (Minor subject: **Biochemistry**) has been approved by the Student's Advisory Committee after an oral examination on the same.

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ABSTRACT

To evaluate the environmental impact on bird population decline, the excreta of nine bird species of agroecosystem was analysed. The feeding habits of nine bird species indicated that blue rock pigeon and eurasian collared dove are grainivorous; common myna, house crow and common babbler are omnivorous; cattle egret and red wattled lapwing are insectivorous and pariah kite is carnivorous.The fresh/ dry excreta of of above birds except pariah kite but including spotted owlet was collected from the roosting , foraging and nesting sites. Excreta varied in texture, colour and shape. Microbial analysis of excreta of blue rock pigeon, rose ringed parakeet, common myna, house crow, cattle egret and red wattled lapwing showed prevalence of the moulds *A. fumigatus* (86.7%), *A. niger*, *A. flavus* (46.7%), *Alternaria* sp. (33.3%) and *Geotrichum* sp. (20%) and bacteria, *E.coli* (73%) and *C. freundii, E. cloacae* and *K. pneumoniae* (64%) . The yeast *Cryptococcus neoformans* was isolated from fresh and dry excreta of blue rock pigeon and fresh excreta of house crow only. The heavy metals analysis in excreta of all nine species included As, Cd, Pb, Fe, Cr, Cu, Ni, Zn and Mn. The blue rock pigeon excreted lowest amount of As, Cd, Cr, Fe, Ni and Mn and cattle egret of Pb, Cu and Zn in the faeces amongst all species studied. However, common myna excreted highest amount of As, Cr, Fe, Ni, Zn and Mn, red wattled lapwing of Cd and Pb and common babbler of Cu. As common myna excreted higher levels of heavy metals which were in toxic range reported for body tissues of birds. It appears to be a protective mechanism for its survival as this bird has been found to be a dominant species in the agrifields of PAU. Only chlorpyriphos was found to be in concentration of 0.046 ppm in house crow and 0.33 ppm in cattle egret amongst pesticide residues(OPs, OCs and synthetic pyetheroids) analysed. The presence of chlorpyriphos in the excreta of the species was correlated with its use for tick control in cattle at the site of collection.

Key Words: Birds, feeding, excreta, moulds, yeast, bacteria, heavy metals, pesticide residues.

Signature of Major Advisor **Signature of the Student**

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ਸਾਰ-ਅੰਸ਼

ਪੰਛੀਆਂ ਦੀ ਘੱਟ ਰਹੀ ਜਨਸੰਖਿਆ ਉਤੇ ਵਾਤਾਵਰਣ ਦੇ ਪ੍ਰਭਾਵ ਦਾ ਅਨੁਮਾਨ ਲਾਉਣ ਲਈ ਖੇਤੀਬਾੜੀ ਵਾਤਾਵਰਣ ਵਿਚ ਪਾਏ ਜਾਣ ਵਾਲੇ ਨੌਂ ਤਰ੍ਹਾਂ ਦੇ ਪੰਛੀਆਂ ਦੇ ਮਲ-ਮੂਤਰ ਦੀ ਛਾਣਬੀਣ ਕੀਤੀ ਗਈ। ਨੌਂ ਤਰ੍ਹਾਂ ਦੇ ਪੰਛੀਆਂ ਦੀਆਂ ਖੁਰਾਕੀ ਆਦਤਾਂ ਦੇ ਅਧਿਐਨ ਤੋਂ ਪਤਾ ਲੱਗਾ ਕਿ ਗੋਲਾ ਕਬੂਤਰ ਤੇ ਘੁੱਗੀ ਦਾਣਾਹਾਰੀ, ਤੋਤਾ ਫਲਹਾਰੀ, ਗੁਟਾਰ, ਕਾਂ ਤੇ ਸੇਰ੍ਹੜੀ ਸਰਬਹਾਰੀ, ਬਦਾਮੀ ਬਗੁਲਾ ਤੇ ਟਟ੍ਹੀਰੀ ਕੀੜਾਹਾਰੀ ਅਤੇ ਇੱਲ ਮਾਸਾਹਾਰੀ ਹਨ। ਇੱਲ ਤੋਂ ਇਲਾਵਾ ਪਰ ਉੱਲੂ ਨੂੰ ਮਿਲਾ ਕੇ ਉਪਰ ਦੱਸੇ ਗਏ ਪੰਛੀਆਂ ਦੇ ਤਾਜ਼ਾ ਅਤੇ ਸੁੱਕਾ ਮਲ-ਮੂਤਰ ਉਨਾਂ ਦੇ ਆਲ੍ਹਣਿਆਂ, ਖਾਣ ਦੀਆਂ ਅਤੇ ਆਰਾਮ ਦੀਆਂ ਥਾਵਾਂ ਤੋਂ ਇਕੱਠਾ ਕੀਤਾ ਗਿਆ। ਮਲ-ਮੂਤਰ ਦਾ ਰੰਗ, ਸ਼ਕਲ ਅਤੇ ਰਚਨਾ ਵੱਖ-ਵੱਖ ਤਰ੍ਹਾਂ ਦੀ ਸੀ। ਕਬੂਤਰ, ਤੋਤਾ, ਗੁਟਾਰ, ਕਾਂ, ਪਸ਼ੂ ਬਗਲਾ ਤੇ ਟਟੀਰੀ ਦੇ ਮਲ-ਮੂਤਰ ਦੇ ਸੂਖਮ ਜੀਵਾਣੂ ਨਿਰੀਖਣ ਤੋਂ ਪਤਾ ਲੱਗਾ ਕਿ ਇਨ੍ਹਾ ਵਿੱਚ ਉੱਲੀ ਇਸਪਰਜੀਲਸ ਫਿਉਮੀਗੇਟਸ (86.7%), ਇਸਪਰਜੀਲਸ ਨਾਈਜਰ, ਇਸਪਰਜੀਲਸ ਫਲੈਵਸ (46.7%), ਆਲਟਰਨੇਰੀਆ ਸਪੀਸ਼ੀਸ (33.3%) ਅਤੇ ਜਿਉਟ੍ਰਾਈਕਮ ਸਪੀਸ਼ੀਸ (20%) ਅਤੇ ਬੈਕਟੀਰੀਆ ਅਸਰਸ਼ੀਆ ਕੋਲਾਈ (73%) ਸੀਟ੍ਰੋਬੈਕਟਰ ਫਰਿਓਂਡੀ, ਐਂਨਟੀਰੀਉਬੈਕਟਰ ਕਲੋਏਕੇ ਅਤੇ ਕਲੈਬਸੀਲਾ ਨਿਮੋਨੀਏ (64%) ਮੌਜੂਦ ਸਨ। ਕ੍ਰਿਪਟੋਕੋਕਸ ਨਿਉਫੋਰਮੈਨਸ ਖਮੀਰ ਕੇਵਲ ਗੋਲਾ ਕਬੂਤਰ ਦੇ ਸੁੱਕੇ ਅਤੇ ਤਾਜ਼ਾ ਮਲ-ਮੂਤਰ ਅਤੇ ਕਾਂ ਦੇ ਤਾਜ਼ਾ ਮਲ-ਮੂਤਰ ਵਿੱਚ ਪਾਈ ਗਈ ਸੀ। ਸਾਰੇ ਨੌਂ ਤਰ੍ਹਾਂ ਦੇ ਪੰਛੀਆਂ ਦੇ ਮਲ-ਮੂਤਰ ਵਿੱਚ ਭਾਰੀ ਧਾਤਾਂ ਤੋਂ ਪਤਾ ਲੱਗਾ ਕਿ ਆਰਸੈਨਿਕ, ਕੈਡਮੀਅਮ, ਲੈਡ, ਲੋਹਾ, ਕ੍ਰੋਮੀਅਮ, ਤਾਂਬਾ, ਨਿਕਲ, ਜ਼ਿੰਕ ਅਤੇ ਮੈਗਨੀਜ਼ ਮੌਜੂਦ ਸਨ। ਇਹਨਾਂ ਸਾਰੇ ਪੰਛੀਆਂ ਵਿੱਚੋਂ ਕਬੂਤਰ ਨੇ ਆਰਸੈਨਿਕ ਕੈਡਮੀਅਮ, ਕ੍ਰੋਮੀਅਮ, ਲੋਹਾ, ਨਿਕਲ ਅਤੇ ਮੈਗਨੀਜ਼ ਅਤੇ ਬਦਾਮੀ ਬਗੁਲੇ ਨੇ ਲੈਡ, ਤਾਂਬਾ ਅਤੇ ਜ਼ਿੰਕ ਸਭ ਤੋਂ ਘੱਟ ਮਲ-ਮੂਤਰ ਰਾਹੀਂ ਬਾਹਰ ਕੱਢਿਆ ਜਦਕਿ ਗੁਟਾਰ ਨੇ ਆਰਸੈਨਿਕ, ਕ੍ਰੋਮੀਅਮ, ਲੋਹਾ, ਨਿਕਲ, ਜ਼ਿੰਕ ਅਤੇ ਮੈਗਨੀਜ਼ ਅਤੇ ਟਟੀਰੀ ਨੇ ਕੈਡਮੀਅਮ ਅਤੇ ਲੈਡ ਅਤੇ ਸੇਰ੍ਹੜੀ ਨੇ ਤਾਂਬਾ ਸਭ ਤੋਂ ਵੱਧ ਮਲ-ਮੂਤਰ ਰਾਹੀਂ ਬਾਹਰ ਕੱਢਿਆ। ਜਿਵੇਂ ਕਿ ਗੁਟਾਰ ਨੇ ਭਾਰੀ ਧਾਤਾਂ ਨੂੰ ਸਭ ਤੋਂ ਵੱਧ ਮਾਤਰਾ (ਜੋ ਕਿ ਪੰਛੀਆਂ ਦੇ ਸਰੀਰ ਵਿੱਚ ਜ਼ਹਿਰੀਲੀ ਮੰਨੀ ਜਾਂਦੀ ਹੈ) ਵਿੱਚ ਮਲ-ਮੂਤਰ ਰਾਹੀਂ ਬਾਹਰ ਕੱਢਿਆ ਹੈ। ਇਸ ਤੋਂ ਇਹ ਪਤਾ ਲੱਗਦਾ ਹੈ ਕਿ ਇਸ ਪੰਛੀ ਵਿੱਚ ਇਹ ਇੱਕ ਬਚਾਉ ਦਾ ਤਰੀਕਾ ਹੈ ਕਿਉਂ ਕਿ ਇਹ ਪੰਛੀ ਪੀ.ਏ.ਯੂ. ਦੇ ਖੇਤਾਂ ਵਿੱਚ ਸਭ ਤੋਂ ਵੱਧ ਮਾਤਰਾ ਵਿੱਚ ਪਾਇਆ ਗਿਆ ਹੈ। ਨਿਰੀਖਣ ਕੀਤੀਆਂ ਗਈਆਂ ਕੀਟਨਾਸ਼ਕ ਦਵਾਈਆਂ ਵਿੱਚੋਂ ਕੇਵਲ ਕਲੋਰਪਾਈਰੀਫੋਸ ਕਾਂ ਵਿੱਚ 0.046 ਪੀ.ਪੀ.ਐਮ ਅਤੇ ਬਦਾਮੀ ਬਗੁਲਾ ਵਿੱਚ 0.33 ਪੀ.ਪੀ. ਐਮ ਦੀ ਮਾਤਰਾ ਵਿੱਚ ਪਾਈ ਗਈ ਸੀ। ਇਹਨਾਂ ਪੰਛੀਆਂ ਦੇ ਮਲ-ਮੂਤਰ ਵਿੱਚ ਕਲੋਰਪਾਈਰੀਫੋਸ ਦਾ ਪਾਏ ਜਾਣ ਦਾ ਸਿੱਧਾ ਸਬੰਧ ਮਲ-ਮੂਤਰ ਦੀ ਇਕੱਠਾ ਕਰਨ ਦੀ ਜਗ੍ਹਾ ਤੇ ਪਸ਼ੂਆਂ ਦੇ ਚਿੱਚੜਾਂ ਦੇ ਕੰਟਰੋਲ ਵਾਸਤੇ ਇਸਦੀ ਕੀਤੀ ਗਈ ਵਰਤੋਂ ਨਾਲ ਹੈ।

ਮੁੱਖ ਸ਼ਬਦ:- ਪੰਛੀ, ਖਾਣਾ, ਮਲ-ਮੂਤਰ, ਉੱਲੀ, ਖਮੀਰ, ਬੈਕਟੀਰੀਆ, ਭਾਰੀ ਧਾਤਾਂ, ਕੀਟਨਾਸ਼ਕ ਦਵਾਈਆਂ।

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ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

CONTENTS

ABBREVIATIONS

AChE	:	Acetylcholinesterase
BHC	:	Benzene hehachloride
cfu	:	Colony forming units
ChE	:	Cholinesterase
CM	:	Carbamate
DDD	:	Dichlorodiphenyldichloroethane
DDE	:	Dichlorodiphenyldichloroethylene
DDT	:	Dichlorodiphenyltrichloroethane
g	:	Grams
GADVASU	:	Guru Angad Dev Veterinary and Animal Science University
HCB	:	Hexachlorobenzene
HCH	:	Hexachlorocyclohexane
kg	:	Kilograms
mg	:	Milligrams
ml	:	Millilitre
OC	:	Organochlorine
OP	:	Organophosphate
PAU	:	Punjab Agricultural University
PCB	:	Polychlorinated biphenyls
POP	:	Persistent Organic Pollutant
ppm	:	Parts per million
SDA	:	Sabouraud dextrose agar
µg	:	Micrograms

SYMBOLS

Ag	:	Silver
As	:	Arsenic
Ca	:	Calcium
Cd	:	Cadmium
Co	:	Cobalt
Cr	:	Chromium
Cu	:	Copper
Fe	:	Iron
Hg	:	Mercury
K	:	Potassium
Li	:	Lithium
Mg	:	Magnesium
Mn	:	Manganese
Ni	:	Nickel
Pb	:	Lead
Se	:	Selenium
Zn	:	Zinc

Chapter I

INTRODUCTION

Birds (class Aves) are feathered, winged, bipedal, endothermic (warm-blooded), egg-laying, vertebrate animals. They have a great diversity of size, form, color and behavior. They have always fascinated man with their intrinsically beautiful plumage, melodious songs and artistic behavior (Shreshtha 2001). The total number of bird species known, as inhabiting the earth today has been estimated as about 8600. If subspecies or geographical areas are taken into account, the figure would rise to nearly 30,000 (Ali 2002). Bird diversity in India is very high compared to other countries owing to diversity in natural habitats and landscapes. The avifauna of India includes around 1301 species, of which 42 are endemic, 1 has been introduced by humans and 26 are rare or accidental (Oleti 2010). The Indian Peacock (*Pavo cristatus*) is the national bird of India. Punjab has a rich bird fauna comprising 328 species of birds (Gupta 2008). The Goshawk (*Accipter gentilis*), is the state bird of Punjab.

Birds constitute an important component of agroecosystems. Birds play an important role in agriculture by feeding on insects, rodent pests of crops, help in dispersal of seeds and in cross pollination in flowers of many plant species (Dhindsa and Saini 1994, Narang *et al* 2000). Among these, insectivorous birds play a major role in managing the insect population in farm lands. A common myna has been seen to have brought food including insects, locusts, caterpillars, grasshoppers etc. 340 times in a day to its nests. A German ornithologist reported that a single pair of tits destroys, annually, at least 120 million insect eggs or 150000 caterpillars and pupae. Over 220 bird species in North America have been reported to consume insects considered as pests in agricultural systems (Kirk *et al* 1996). A recent meta-analysis of bird enclosure studies from tropical forests and agro-forests similarly concluded that higher bird richness is associated with significantly reduced arthropod densities (Van Bael *et al* 2008). Biocontrol by birds was also reported in apple orchards where *Parus major* reduced caterpillar damage (Mols *et al* 2005). Birds such as drongos, mynas, shrikes and kingfishers feed on insects and caterpillars in the rice fields during daytime. In the night, different species of owls prey upon rodents that contribute to crop loss, especially in food grain cultivation. Egrets, herons, water hen and snipes feed on worms, moths and caterpillars as well as harmful soil organisms in rice agrifields. Some studies have shown that crow pheasant (*Centropus sinensis*) is very good in controlling red palm beetle, which is a critical pest in coconut palms. Some of the birds such as oriental white-eye, sunbirds, drongos, flycatchers, magpie robin also help in pollination in various crops (Vishnudas 2007).

Agriculture provides a concentrated and highly predictable source of food to birds. Bird community in agricultural lands is characterized by overdominance of only a few grainivorous and omnivorous species while the rest of species are represented in very small numbers, some of which are rare. Agricultural landscapes in India, especially in the intensively cultivated areas like Punjab, have a number of fish, dairy, poultry and honey bee farms interspersed among crop fields which provide additional food to birds in the form of fish, bees, animal feeds, tree fruits, seeds, nectar etc. The very abundant and widespread grainivorous and omnivorous species come in conflict with man's interest of increasing agricultural production by inflicting economic injury to crops, fruits and stored grains. Insectivorous and carnivorous species are considered to be useful to agriculture since they keep a very potent check on populations of insect and rodent pests of crops. These birds, however are very less abundant than grainivores and omnivores.

The dual role of birds in agriculture is very well known (Ali 1949, 1971). Avian management includes both conservation of useful species and control of pest birds. The assessment of environmental impact of various control techniques is also an essential component of management. The today's explosive world, has led to ecological explosions. The rapidly changing technologies like the use of chemicals (pesticides) in agriculture since 1945 for the purpose of controlling various pests, has given rise to concern about their effects on man and his environment as well as the effects on birds in particular, which has been intensively studied in U.K. and U.S.A. Pesticides cause significant bird mortality each year. One well known estimate (Pimentel and Acquay 1992) suggested that more than 670 million birds are directly exposed to pesticides each year in U.S. farms alone, 10% of which or 67 million birds die as a result.

Birds provide an early warning of environmental problems. Healthy avian populations are indicators of ecological integrity. Decline in avian populations shows a collapsing ecosystem (US FWS 2002). Agricultural areas in India probably experience the most heavy and indiscriminate use of chemical pesticides leading to direct and indirect mortality of predatory and frugivorous birds (Dhindsa *et al* 1986). The birds must also have been contaminated with their residues. Heavy metal toxicity is of major concern in wild and captive avian species (Aizenberg *et al* 2006). Birds of prey being at the top of the food chain are the most likely victims of pesticidal and heavy metal contamination. It has been reported that continued application of pesticides containing heavy elements like cadmium and mercury leads to the accumulation of these dreadful elements in the body of the birds as they feed on affected creatures like small fishes, shells etc. Use of dichlophenol, as an anti inflammatory drug in livestock, has made the Indian white backed vulture *Gyps bengalensis,* a bird of sky to a bird of captivity during the past five years. When it comes to birds of higher levels in the food chain, like raptors, owls and also egrets, they develop different abnormalities like, laying of shell less eggs, some times, thinner eggshells. The eggs having thinner eggshell cannot be incubated by the birds. This in turn affects the reproductive capacity of the birds too (Vishnudas 2007). Microorganisms (*Escherichia coli, Staphylococcus epidermitis*, *Isospora lacazei, Candida* sp.), heavy metals, pesticides and food shortage were investigated as possible causes of embryo and nestling deaths of sparrows *Passer domesticus* (L.) and *Passer montanus* (L.) (Pinowski *et al* 1994).

Mortality of these and other birds due to toxic chemicals also needs to be assessed because several crops and insects contaminated with the insecticide are taken by avian species. The assessment of the effects of these compounds upon birds requires the integration of various types of information e.g. population trends of various bird species in recent years, the results of chemical analysis of tissues and eggs, laboratory studies of the pharmacological and toxicological effects of these compounds. There has hardly been any information on residue analysis and other toxicological aspects on birds of agricultural habitats. However, significant decline has been reported in the population of a number of bird species, but the reasons for the same are not properly correlated. This may perhaps be because of habitat destruction due to intensive agriculture and urbanization. Moreover, the impact of environmental contaminants like agricultural (pesticides) and industrial (heavy metals) pollutants in our water bodies like Buddha Nullah as its deadly water is badly affecting south-western areas of Punjab still needs to be assessed. Since birds have no boundaries such as protected areas or agricultural lands, their conservation should be looked into while managing agricultural landscapes. The local extinction of the vultures, from the Punjab plains, has brought to light the importance of birds as scavengers since many road kills remain for days on the road, waiting to be picked up by the municipal committees, as other scavenger birds like pariah kite, mynas and crows, in spite of their increased populations, are no match for the vultures and their keen sense of detecting carcass (Joshua and Ali 2011) .

Birds are more susceptible than mammals to many pesticides, and this may be due to relatively inefficient systems of detoxification (Walker 1978). Schafer (1972) determined the acute oral toxicity of 61 pesticides and other chemicals, and obtained LD_{50} values of 110 mg/kg for rats, 48 mg/kg for starlings (*Sturnus vulgaris*) and 15 mg/kg for red-winged blackbirds (*Agelaius phoeniceus*). The differences between species were statistically significant. The hepatic microsomal enzymes monooxygenase and epoxide hydrolase are more active in mammals than in birds (Walker 1980). Since hepatic microsomal monooxygenase has an important role in the detoxification of many liposoluble pesticides, its low status in birds may contribute to their high susceptibility to certain chemicals. The high toxicity of organophosphates to birds, such as diazinon (Machin *et al* 1976) and pirimiphosmethyl (Brealey *et al* 1980) has been correlated with relatively low 'A' esterase activity in avian plasma. The low activity of certain detoxifying enzymes in birds may result in the efficient accumulation of certain pollutants that are slowly metabolized and are present at low levels in their food. The understanding of the relationship between metabolism of pesticides and their toxic action in birds is very limited. There is little information on the *in vivo* metabolism of pesticides and the relative importance of urine and bile as routes of excretion for these compounds. Chipman and Walker (1981) have reported that metabolism and excretion of HCE is slower in the pigeon than in the male rat, thus providing further evidence for the limited capability of the species to perform certain detoxifying transformations and thus making it undectable in faecal matter.

In the latest assessment in 2011 by Birdlife International, 1,253 species are considered threatened with extinction (i.e. in the categories of critically endangered, endangered and vulnerable). An additional 843 species are considered Near Threatened and four are Extinct in the Wild, giving a total of 2,082 species that are urgent priorities for conservation action. Of the threatened species, 189 species are considered Critically Endangered and are therefore at extremely high risk of extinction in the wild. Unfortunately, India is the third among the countries having the largest number of rare and threatened species followed by Brazil and Indonesia. Habitat loss is the greatest threat to most of the Indian birds (Rahmani 2008). Nearly 12 per cent of India's birds are facing extinction. The Bird Life International and the IUCN (International Union for the Conservation of Nature) have listed 12 species in India as 'critically endangered'. These are in dire need of ICU (intensive care unit) (Dandapat 2010).

According to Wildlife Protection Act 1972, the capturing and killing of birds is legally banned by Govt. of India, therefore any analytical studies on the tissues of these animals are beyond the reach of scientists working in this area. Thus, an excreta of bird is the only source, which if analyzed can give an assessment of the harmful impact of environmental contaminants on these animals. Studies regarding the chemical composition and characterization of faecal material are important, since modifications of chemical processes in a diseased organism usually induce modifications in the quantity and composition of faeces. In certain cases, this contributes to the presence and subsequent detection of biological and chemical substances, absent under normal conditions. The composition of normal faeces depends at large on the quality and composition of the food consumed.

As environmental impact analysis is an essential component of all management strategies for birds and nothing has been done in this direction in India, it was planned to conduct analytical studies biologically and chemically on the excreta of birds with different feeding habits. The study may give information about the environmental impact on alterations in the metabolism of birds with different feeding habits thus giving clues for their use in management strategies for conservation of birds and preventing decline in population of useful birds. Therefore, the present studies were planned with the following objectives:

(1) To study the feeding behavior of common birds of agroecosystems

(2) To collect fresh and dried excreta of omnivorous, grainivorous, carnivorous and predatory birds.

(3) Microbial analysis of bird excreta.

(4) Estimation of heavy metals in bird excreta.

(5) Analysis of pesticide residue/ metabolites of commonly used pesticides in bird excreta.

Chapter II

REVIEW OF LITERATURE

2.1 Birds in Agriculture

India is basically an agricultural country with different geographic features and with the most variable climatic conditions. About 43% of India's geographical area is used for agricultural activity. A variety of cereals, oil seeds, pulses, vegetables and horticultural items are being cultivated in the country. According to a recent estimate by the Union Ministry of Agriculture, pest-related damages result in an estimated loss of Rs. 50,000 crore annually in agricultural production in the field and at storage in India. It has been reported that nearly half of the potential yield of rice is lost due to pests (Ali *et al* 2010).

Birds constitute an important component of agroecosystems. Mixed flocks of birds belonging to several species feed in areas of agricultural crops and fruit trees. Most of the bird species play a useful role in agriculture by having a potent check on insect and rodent pests. Although 1% of the world's bird species primarily prefer agricultural areas, nearly one-third of all birds occasionally use such habitats (Sekercioglu *et al* 2007), often providing important ecosystem services, such as pest control, pollination, and seed dispersal (Greenberg *et al* 2000, Perfecto *et al* 2004, Sekercioglu 2006, Van Bael *et al* 2008). On one hand, birds damage the crop at various stages, beginning from sowing to the storage state; while on other hand they benefit the crop by feeding voraciously on the pest species and in turn controlling the pest population (Gokhale 1992). More than 37% of the total 3000 reported species of birds in world exclusively depend upon insects for food (Martin and Li 1992, Ali 2002). There is an increased evidence that insectivorous birds increase the fitness of the plants on which they forage, e.g. by significantly reducing the number of insects on the plants (Solomon *et al* 1976, Holmes *et al* 1979, Gradwohl and Greenberg 1982, Campbell *et al* 1983, Lyon *et al* 1983, Joern 1986, Atlegrim 1989, Fowler *et al* 1991, Moore and Yong 1991, Bock *et al* 1992).

India is bestowed with rich heritage of avian diversity consisting of 85 per cent, of them partially or fully insectivorous in nature in different agro-climatic regions of the country (Ali and Dillon 1983). Several species of insectivorous birds have been found to feed on insect pests of crops including gram pod borer, *Helicoverpa armigera* (Chakravarthy 1988) which are known to reduce the larval population to the extent of 84 per cent in Punjab. Jungle babbler (*Turdoides striatus*), a widely spread sub-tropical insectivorous passerine is considered beneficial to agro-ecosystem, as they devour voraciously on insect matter especially *Helicoverpa armigera,* the gram pod borer, a notorious pest infesting and causing heavy loses to crops like pigeon pea (*Cajanus cajan*) which is a vital crop of semi-arid tropical and subtropical farming system, providing high quality vegetable protein.

Helicoverpa is known to feed on flowers, pods, and seeds and is the most important biotic constraint affecting pigeon pea yields (Bharucha and Padate 2010). Among the predatory birds, black drongo, house sparrows, blue jays, cattle egret, rosy pastor and mynah have been commonly recorded predators on large number of *H. armigera* and lepidopteran insects on chickpea, pigeonpea, and groundnut crops (Gokhale and Ameta 1991). The white grub (*Holotrichia* sp: Scarabidae) is an important subterranean pest damaging root systems of several crops (Parasharya *et al* 1994). Experiments conducted during 1985 and 1986 showed that at least 14 species of birds picked up the grubs exposed during ploughing operation. The important bird predators were mynas, *Acridotheres tristis* (Linnaeus) and *Acridotheres ginginianus* (Latham); crows, *Corvus splendens* (Vieillot), *Corvus macrorhynchos* (Sykes); drongo, *Dicrurus adsimilis* (Hodgson) and cattle egret , *Bubulcus ibis.* The birds were found to reduce 45 to 65% grub population during 3 subsequent ploughings. The plant stand of second crop raised in bird exposed field was higher in experimental plot compared to the control. Insectivorous birds indirectly defend oil palms (*Elaeis guineensis*) from herbivorous insects (Koh 2008). An attempt to identity avi-wealth and to assess its status as predators of mainly underground pests of potato was made during September, 1996 to March, 1997. This resulted in identification of 17 avi-predators useful in reduction of regional potato pests. They were noticed to pick-up insects during all field operations even from standing crops but particularly during irrigation and soil disturbance at mechanical harvesting, interculture and field preparation operations. They were seen picking up grubs, caterpillars, worms, beetles and other insects. Most active avi-predators were common myna, house crow, black drongro, red wattled lapwing, large pied wagtail, cattle egret and pied myna (Sharma 1998). The common mynas are potential hunters of insect pests and can therefore be used for controlling insect pests in crop fields and fallow lands (Ali *et al* 2010). However, some grainivorous bird species, having adapted to agricultural habitats and increased in numbers, are conflicting with goals of agricultural production by inflicting economic losses to crops, fruits and stored grains.

A number of bird species are pests of agricultural crops (Ward 1965, Murton and Wright 1968, Grist and Lever 1969, Dyer and Ward 1977, Besser 1978, Mehrotra and Bhatnagar 1979, O'Conner and Shrubb 1986). Bird pests are considered as a potential threat to crop production due to their habit of damaging various agricultural crops. They cause economic losses in a variety of crops like wheat, maize, sun flower, groundnut and citrus etc as reported by many farmers (Brooks and Ahmed 1990, Khan and Ahmad 1990). Patel (2011) observed that blue rock pigeon and Indian ring dove are grainivorous birds and they damaged sown seeds of sorghum and pearl millet while the rose-ringed parakeet was the major bird pest of millet crops (Bajara and Jowar) in Patan district of Gujarat. They feed on millet grains during the grain maturity stage, causing a considerable loss. House crows and jungle crow were also seen feeding on millet grains during the sowing and germination stage as well as at the grain maturing stage. Baya weaver performed dual role as it was found to feed upon larvae of insects on millet crops but damaging leaves of sorghum for nest construction. Common myna, bank myna, common babbler and rosy pastor were observed feeding mainly on insect pests of millet crops (Patel 2008). Mynas may consume grapes, apricots, apples, pears, strawberries, gooseberries and other fruit, damaging food crops (Brochier *et al* 2010). Mynas are omnivores (Sengupta *et al* 1976) and could potentially compete with a large range of indigenous species for food and also can cause severe damage to the agricultural crops (Narang *et al* 1984) in the local community.

Bird management involves both the conservation of useful species and control of pests (Dhindsa and Saini 1994). Birds of agricultural system are one of the most threatened species of birds mainly due to their sharp population decline in the recent decades. Habitat intensification resulting for more production, agricultural practices has been proposed as a major cause for this decline. Intensification of agricultural practice, such as crop specialization, pesticide use and elimination of natural and semi-natural habitats have been proposed causes underlying the reported decrease in the faunal quality of agricultural habitat (Bharucha and Padate 2010). Agroecosystems of Punjab, for the past many decades, have been subjected to tremendous changes owing to massive deforestation, intensive agriculture and excessive use of pesticides alongwith rapid urbanization and industrial growth which in turn has affected the avian fauna resulting in reshuffling of many species of birds.

Seeds are a major food of many birds and comprise practically the entire diet of some species (Holland *et al* 2006). Viable seeds may be transported away from the cultivated field in three possible ways: (1) physically on a bird's feet or feathers; (2) by remaining in the esophagus/crop or gizzard and being relevant in the fate of the carcass; or (3) passing intact through the digestive tract and being deposited as faecal material (Cummings *et al* 2008). Numerous studies have documented that birds disperse a wide variety of seed types (McAtee 1947, Van der Pijl 1982). The physical properties of seeds influence their passage through the digestive tract (Powers *et al* 1978, Traveset 1998). Small seeds (1–3 mm) with hard seed coats pass through the digestive tract of many species of birds. In some cases the digestive action enhances the germinability of seeds by softening or removing the seed coat. Studies have shown that seeds, which pass through the digestive systems of the birds, are more likely to germinate and sprout earlier (Midya and Brahmachary 1991).

Avian frugivores are considered as the most important seed dispersers in most ecosystems (Herrera 1995, Stiles 2000). Frugivorous birds are a priority for conservation. They are experiencing the transformation of natural habitats to agro-ecosystems worldwide and some are taking advantage of agricultural production of fleshy-fruited plants. However, the mechanisms through which some birds are able to thrive in agricultural landscapes while others become extinct are poorly known (Rey 2011). Balasubramanian *et al* (2011) reported that birds constituted the principal seed dispersers of *Santalum album* which is a medium sized evergreen tree found in dry forest tracts of the Deccan Peninsula, where the major sandal growing tracts are located in Karnataka and Tamil Nadu. Sandal is also distributed in parts of Maharashtra, Andhra Pradesh and Kerala. The species was introduced to several areas of central and northern India, where it has naturalized and spread. *Santalum album* is a Vulnerable (IUCN 2010) and threatened species in southern India (Ravikumar *et al* 2000). Birds beneficial to sandalwood dispersal and regeneration are koel, common myna, brahminy starling, brown-headed barbet, white-headed babbler and Indian grey hornbill. These species visited the fruit crop more frequently and swallowed the fruit wholly. Hence, these species could be considered as major seed dispersers. Asian koel seems to have preference to sandal fruits. Efforts need to be undertaken to provide a healthy habitat for the seed dispersing bird species such as koel, as the population of sandalwood tree is drastically dwindling in the wild.

2.2 Food and Feeding Habits of Birds

Food and feeding behavior of different bird species of a region is first and foremost requirement to assess their economic status and to initiate work on bird management in that region. Unfortunately, only limited information is available on the feeding ecology of Indian birds. As a sequel, the economic status of many bird species remains yet to be defined accurately. The food of birds in general is of three kinds: (i) grains, seeds and fruits, (ii) green vegetation of the crop plants and grasses and (iii) insects, other arthropods and rodents etc. found in the soil, crops and other plants (O'Connor and Shrubb 1986). Birds of agricultural areas include grainivores, frugivores, insectivores, carnivores, nectarivores and omnivores but there is overdominance of only a few grainivorous and omnivorous species while rest of species are represented in small numbers while the rest are rare. There are about 1200 species of birds occurring in the Indian subcontinent, at least 150 of them are reported to feed on seeds and fruits of different crops and only 25 species (about 2.1%) inflict serious damage to crops during vulnerable growth stages. Crop fields attract many birds from sowing to grain maturity stage (Bhalodia *et al* 1997). During the germination certain biochemical changes occurs which increase the nutritive value of seeds (Singh and Benarjee 1955, Chen *et al* 1975) which may provide excellent nutrition for seed eating birds.

A study carried out in South India on the Indian blue peafowl, *Pavo cristatus*, a grainivorous bird feeding on grains and seed crops, assessment of interrelationship of peafowl and agriculture and determination of its pest status revealed that the peafowl had an innate preference for grains like paddy as their bulk food and secondarily finger millet (ragi), pearl millet (sorghum) and sesamum and that the crops were damaged only at the ripening stage. Damages caused by these birds can be reduced by using different lethal and non-lethal methods (De 2005). Khan *et al* (2007) reported that crow is a primary consumer and omnivorous in nature, foraging in small flocks throughout the day on farm crops, livestock farms, and in residential areas. The crow *carte du jour* mainly comprises the kitchen refuse, fallen fruits in the gardens, and stored grains. The damage proportions recorded for the crow are of important food crops, thereby, indicating that the crow has acquired the status of a serious avian pest (Berruti 1990). According to Dhindsa *et al* (1991), the damage recorded by crow to sprouting sunflower was 70% in an unguarded situation at Ludhiana, India. It was evident that more damage occurred on the seedling stage than that at the mature stage. Losses to almonds by crows in Himachal Pradesh, India have been reported by Bhardwaj (1991). According to him, varying flocks of crows (25 to 30) through frequent visitations in the morning hours and again in the afternoon, destroyed about 55% of the almonds. Besides crows, other avian species viz. the rose-ringed parakeet (*Psittacula krameri*), house sparrow (*Passer domesticus*), common myna (*Acridotheres tristris*) and rosy starling (*Sturnus roseus*) are also regarded as pestiferous and as such, cause substantial economic losses (Shafi *et al* 1986). House crow destroys the food crops and the wheat grains in good numbers particularly at the post harvest level, and, therefore, directly competes with man for the food resources, but also causes significant economic losses (Dilshan *et al* 1982, Gourami 1985). Maize is one of the preferred food items for the house crow. The intensity of depredations on the maize has been reported to be widespread by the rose-ringed parakeet, while a little lesser intensity of damage has been reported by the crows, sparrows and the common mynas (Khan and Ahmad 1983, Malhi and Brar 1987, Gupta *et al* 1998). Brooks and Ahmed (1990) stated that crows invaded fresh sown maize, barley and wheat fields and dig out the emerging seedlings. The late sown fields were usually the worst hit. Damage was more sever to cropping fields nearest to the crows roosting sites. They also damaged ripened crops, especially maize and groundnut. Maize kernels were attacked worst in milking stage. The authors reported that parakeet was very destructive to maize, wheat, sunflower, sorghum and other grain crops. The parakeet also damaged the soft fruits such as guava, mango, orange and loquat wearily. House crows are known to be useful in removing pests. They were, in fact, introduced in Malaysia and Oman to control caterpillars and livestock ticks. House crows are omnivorous scavengers with a wide-ranging and opportunistic diet. They eat absolutely everything that is edible, and some that may not even be so. The crow feeds largely on refuse around human habitations, carrion, small reptiles, insects, small invertebrates, eggs, nestlings, plants, grain and fruits etc. Being closely associated with people (no populations are known to live independently of man), the crow takes advantage of scavenging opportunities provided by discarded food items and refuse dumps. This is particularly true in urban environments where other food sources like fruits, grain, insects, eggs etc. are limited (Sen 2011).

Columba and *Streptopelia* are ground feeders and predominantly grain-eaters, although quite often they invade diversified food-niches (Bhattacharyya1994). The food of the blue rock pigeon chiefly consists of grains of rice, maize, millet and other cereals, pecked from the ground. This species also feeds on banyan figs and other berry-like fruits and sometimes insect eggs and larvae. Besides grains of paddy, wheat, millet, jowar (*Sorghum* sp.) and other cereals and pulses, very often feeds on seeds of mustard, linseed and various weeds. Rana (1991) reported that the ring dove changes its food-habit quite frequently, depending on the availability of food; in the monsoon, the birds also feed on insects.

Common myna often feeds on different food items depending on the time of year. This is due to the change in the availability of certain foods (Dhandhukia and Gadhvi 2010). Ali *et al* (2010) studied the regurgitated pellets of common myna at two villages Mannampandal and Thiruvalangadu of Cauvery delta region in Nagapattinam District, Tamil Nadu, India, between 2005 and 2006 which revealed the insect prey remains: Coleoptera - beetles (mandibles, elytra and leg fragments), Hemiptera – bugs (H-shaped tergal plates, mouthparts and leg fragments), Hymenoptera – bees, ants and wasps (mouthparts, leg fragments and wing fragments), Orthoptera - grasshoppers (raptorial leg fragments and mandibles), Diptera - flies (antennae, eyes and wings), Odonata – dragonflies (wings, leg fragments and head capsules) and Lepidoptera – butterflies and moths (wing scales and proboscis). Insects were the main food of the common myna, constituting 92.40% (6,869 individuals) of the total number of prey items. Among insects, beetles (coleoptera) were dominant and comprised 24.17% (1003 individual remains) followed by grasshoppers (19.88%), bugs (16.33%) and bees, ants and wasps (13.49%). The other insects constituted <10% in the diet of the common myna. Beetles were predominant prey items in both the habitats and constituted 24.23% and 24.10% respectively. Other major insect preys found in the pellets were grasshoppers (20.44% in the agricultural field and 19.17% in the non-agricultural field), bugs (17.21% and 15.22%) and hymenopteran insects (14.60% and 12.09%). The common myna is a ground forager, which caught varieties of beetles, grasshoppers, ants, butterflies, etc. on the ground of the agricultural fields. Overall, common myna spent most of the day scanning (51.58%), with significantly less time spent on feeding (23.62%). Earlier, Mathew *et al* (1978) reported that common myna was more abundant in rice fields and took many ground beetles and 53% of the stomach samples consist of harmful insects of the order orthoptera, isoptera, coleoptera and hymenoptera.

A few grainivorous and omnivorous bird species have been able to harvest energy and reproduce very efficiently in agricultural habitats leading to their large population build-ups. Gut contents of large grey babbler revealed that this species is mainly insectivorous (Toor and Saini 1986). Analysis of the guts of 402 house sparrows revealed that the major portion (78.94%) of the diet was plant matter and grit represented 20.34%, animal matter only 0.68% of the food and 6% of the contents could not be identified (Toor *et al* 1986).

Parakeets are completely vegetarian, feeding upon plant materials. Gut contents recorded in adult birds contained maize grains, groundnut, guava fruits, mango, jamun, anthers and tree barks in different crop seasons (Kushwaha and Kumar 2007). The study by Hussain *et al* (1991) revealed thar flocks of parrots were observed to attack the guava fruits. The crows also attacked the fruits with their beaks and rendered them unfit for marketing. Parakeets prefer the food sources closer to their nest sites for effective predation (Karim 1987). The rose-ringed parakeet, by virtue of its utter astuteness and opportunism, appears to have benefited greatly, possessing a wide feeding niche, and consuming cereals, orchards, oil-seeds and stored. Parakeet is a wasteful feeder and discards or destroys partially consumed food items (Ali and Ripley 1988). Parakeets feed the maize crop at the milky and mature stages, removing the outer leafy foliage and consume the grains (Ramzan and Toor 1988). The list of cultivated cereals and other food crops consumed by the parakeet is wide (Ali and Ripley1982, Paton *et al* 1982, Chakarvorty *et al* 1998, Khan 2002). Although the parakeet possesses a wide feeding niche, yet it more explicitly attacks the oil seed crops viz. sunflower, brassica and canola, apart from a variety of orchards (Khan *et al* 2006). A loss of 25- 100% to brassica, mangoes, guava and sunflower by parakeet was reported by Prasad and Verghese (1985) in India. Saini *et al* (1993) analyzing the gut contents of the rose ringed parakeets for one year showed that it consisted of cereals (45%), tree orchards(38%) and oilseeds (16%).

The dietary composition of the white-breasted kingfisher, *Halcyon smyrnensis*; the small bee-eater, *Merops orientalis* and the black drongo, *Dicrurus macrocercus* was studied between 2005 and 2006 in Nagapattinam District, Tamil Nadu, India by analyzing regurgitated pellets. The analysis revealed that the white-breasted kingfisher preys mainly on arthropods (83.40%) and less on vertebrates; seven orders of insects were identified, with Coleoptera, Hemiptera, Hymenoptera and Orthoptera being predominant. The small bee-eater diet consisted of Coleoptera (22.3%), Hymenoptera (20.8%), Hemiptera (14.1%), Orthoptera (12.6%), Odonata (10.7%), Lepidoptera (10.4%) and Diptera (8.6%). Beetles were also found to be the most frequent prey (23.7%) in the diet of black drongos, followed by Hemiptera (21.6%), Orthoptera (19.3%), Hymenoptera (14.4%), Lepidoptera (7.5%), Diptera (6.8%) and Odonata (6.0%) (Asokan *et al* 2009). Sharah *et al* (2008) reported that the cattle egrets (*Bubulcus ibis*) were observed to forage for insect-preys in various and different types of ecosystems, such as farm-lands, grasslands, dry river and lake beds and banks, along the roads and when moving along side grazing livestock, picking various species of insects disturbed by these animals. The birds forage as single, in pairs and in flocks, searching, running and flying after preys to catch them. Large vertebrate preys caught were killed using the sharp robust, long bill by smashing them on the ground, tore them into shreds and the pieces ingested.

The feeding behavior of the cattle egrets, which took orthopterans and other invertebrates taking the major insect preys were mostly of insect–pest of agricultural crops with 88.7% pest status. The cattle egrets, restricted in migration and nomadic behaviour (Maddock 1990) are insectivorous with almost 98% reliance on orthopterans in terms of prey occurrence in the guts. Nesting cattle egrets parents forage and capture any available insect to feed their off-springs (biparental care), thus making it easier to infer the diet of adult egrets from those of the chicks (Jenni 1973). Sharah *et al* (2008) reported that seasonal changes, affect insect–prey population, with many species of insect prey disappearing after the rain. Such seasonal changes, which also make scarce the availability of seasonal insect-prey species, influence the prey species (types) consumed by the cattle egrets. The absence of some wet season predilected insects e.g., the elate Isopterans, suggest that the presence of drier conditions could not favor the multiplication of such insects. Siegfried (1971) reported similar situation when 85% of the gut contents of the egrets were earthworms in winter, but none was found in summer periods. Cattle egrets which once feed in close association with grazing cattle have now shifted themselves behind tractors in inundated agricultural fields (Mukherjee 2000).

Pariah kites (*Milvus migrans*) often gather in large communal roosts and breeding colonies. Large aggregations also occur where food is abundant and during migration. Their highly gregarious and opportunlstic foraging behavior leads them to eat the most abundant and available prey, especially slow-moving and injured animals as well as food obtained by scavenging (Shiraishi *et al* 1990). As scavengers pariah kites have been recorded in large numbers on waste accumulations generated by human activities, including rubbish dumps, markets, fishing-ports and abattoirs all over their world range (Pomeroy 1975, Shiraishi *et al* 1990).The rats and mice, which are forced to come out of their burrows when the harvested wheat and sugarcane fields are flooded with irrigation water, are caught by pariah kites and the house crows (Beg *et al* 2010). Among the nocturnal birds of prey, two species of owl viz. the barn owl (*Tyto alba*) and the spotted owlet (*Athene brama*) are important predators of the agrosystems of the Punjab (Beg *et al* 1990, Shah *et al* 2004, Mahmood *et al* 2007).

2.3 Microorganisms and Birds

The complex relationships between birds and microorganisms are increasingly becoming the subject of ecological research (Maul *et al* 2005). Microbial species can interact with birds in many different ways. Some are commensals, living *in vivo* as part of the normal feather or gut flora without apparently affecting their host. Some are avian pathogens, either obligatorily (e.g., *Chlamydia psittaci*) or opportunistically (e.g., *Pseudomonas aeruginosa*). Other microbes, particularly fungi such as *Cladosporium* and *Epicoccum*, have the potential to be allergens (Hubalek 1978). Both pathogenic and allergenic species can act to reduce fitness, making individuals more susceptible to competition and predation, while severe infections/reactions are significant causes of mortality (Nuttall 1997). Conversely, the presence of microbes can be beneficial; for example, *Enterococcus faecium* has been found to increase the fitness of pied flycatcher (*Ficedula hypoleuca*) nestlings (Moreno *et al* 2003), while *Eupenicillium javanicum* contains the cyclic depsipeptide, eujavanicin A, the antifungal properties of which are effective against *Aspergillus fumigatus*, a cause of avian aspergillosis (Nakadate *et al* 2008).

2.3.1 Moulds

Fungi are important causes of diseases in wild birds and other species. Three basic types of disease are caused by these agents: mycosis, or the direct invasion of tissues by fungal cells, such as aspergillosis; allergic disease involving the development of a hypersensitivity of the host to fungal antigens and mycotoxicosis, which results from ingestion of toxic fungal metabolites. Mycosis and allergic disease may occur together, especially when the lung is infected. Allergic disease is not well studied in wild birds. Most disease-causing fungi are commonly found within the normal environment of hosts that may become diseased. Host resistance is the main determinant of whether or not disease will occur. Opportunistic infections often result when birds and other species are immunosuppressed, when their mechanisms for inflammatory response are inhibited, or when they experience physical, nutritional, or other stress for prolonged periods of time. Newborns do not have fully functioning immune systems and are, therefore, especially vulnerable to mycosis as are very old animals that are likely to have impaired immune systems. Inhalation is the primary route for exposure to most fungi-causing mycosis. Aspergillosis is the primary mycosis affecting wild birds. Candidiasis is a less common mycosis of wild birds and other species, but it differs greatly from aspergillosis by being transmitted by ingestion. These two diseases are the primary mycoses of wild birds. The stress appears to be an additional factor in the development of fungal infections (Balseiro *et al* 2005). This stress can be associated with captivity, inadequate management, prolonged treatment with antimicrobials, or other debilitating conditions. It can also be associated with a certain form of physiological stress which may be greater at certain times of the year, such as during the breeding season or in winter when environmental conditions are more severe (Redig *et al* 1980, Bauk 1994, Cork *et al* 1999). There is evidence that the occurrence of this stress needs to be concurrent with the inhalation of spores to develop a fungal disease (Dixon *et al* 1989, Redig 1993, Bauk 1994).

Aspergilli are saprophytic moulds that are closely associated with agriculture and other human activities that make nutrients available to fungi. Aspergillosis is a major cause of morbidity and mortality in birds (Tell 2005). Aspergillosis is a respiratory tract infection caused by the genus *Aspergillus*, of which *A. fumigatus* is the primary species responsible for infections in wild birds (occasionally *A. flavus* and infrequently caused by *A. terrus, A. glacus A. nidulans, A. niger A. amstelodami, A. nigrescens*). McDiarmid (1955) has shown that free-living wild birds become infected with *Aspergillus fumigatus* and other *Aspergillus* sp. which produce aspergillosis. *Aspergillus fumigatus* has been determined as the most pathogenic among the causative agents of aspergillosis with widespread resistant spores present in the natural environment (Raines *et al* 1956, Reece *et al* 1986, Akan *et al* 2002, Richard 1997). A unique feature of avian aspergillosis is the presence of reproductive phases of *Aspergillus* sp. in tissues. This has been reported for the asexual phase of *A. fumigatus* (Richard *et al* 1980) and both the asexual and sexual phases of *A. nidulans* (Stedham *et al* 1968). Potential reasons for this finding include the presence of cavernous air sacs, the warm core body temperature of birds (range=38-45°C) that might promote conidial germination and birds sensitivity to gliotoxin that might result in tissue necrosis and thus provide a nutrient rich environment (Friend 1999). Aspergillosis is one of the most frequent infectious diseases affecting stressed and immunosuppressed animals (Briggs *et al* 1996, Carrasco *et al* 2001, Friend 2001). Anatomic characteristics that might predispose birds to this disease include a lack of an epiglottis for preventing particulate matter from entering the lower respiratory tract, lack of a diaphragm resulting in the inability to produce a strong cough reflex and a limited distribution of pseudostratified ciliated columnar cells through the respiratory tract. Cellular characteristics that might predispose birds to respiratory aspergillosis include a lack of surface macrophages for phagocytizing A*spergillus* sp. conidia and dependence on heterophils that use cationic proteins, hydrolases, and lysozymes rather than myeloperoxidase and oxidative mechanisms for killing fungal hyphae (Klika *et al* 1996, Harmon 1998). The ubiquity of the organism suggests that birds may be carriers of the fungi but do not develop overt disease unless stimulated by a decreased resistance of the host elicited by some stress such as an infectious disease, a toxicant, or malnutrition. Experiments indicated that birds subjected to a handling stress had higher levels of corticosteroids (Fowler *et al* 1995, Fowler 1999, Deem 2003, Park 2003, Balseiro *et al* 2005) which can affect the immune system in many ways (Griffin 1989). The number of colonies for establishing a definitive diagnosis is usually between one and four; the recovery of large numbers carries a severe prognosis. A simple colony is significant and should not be disregarded as an incidental finding (Redig 1993). A wide variety of birds (waterfowl ,gulls, crows, raven, raptors, songbirds, upland gamebirds blackbirds, cowbirds, grackles, herons and shorebirds) have died of aspergillosis and probably all birds are susceptible to it. Young birds appear to be much more susceptible than adults (Friend 2001).

Faecal material and debris samples from 18 nests of Philippine red vented cockatoos was taken by Burr (1981) and plated on Sabouraud Agar. Out of these 18 samples, 16 proved positive for *Aspergillus fumigatus.* The pigeon frequently has been associated with pathogenic and saprophytic fungi (Emmons 1960).

With regard to other kinds of moulds, the order Mucorales includes a number of saprophytic fungi associated with an underlying diseased condition. The most common infection route is by inhalation of the spores (Reavill 1996). Mucor infections have been reported as possible aetiological agents of meningoencephalitis in birds (Orcutt and Bartick 1994), they have also been isolated from a military macaw (*Ara militaris*) with severe bronchopneumonia (Dorrestein *et al* 1985) and from a penguin with pneumonia and air sacculitis. There are a few documented cases of diseases caused by *Penicillium,* described as the aetiological agent of a localized beak infection in a macaw, *Ara ararauna* (Bengoa *et al* 1994), associated with feather lesions and found in the lungs, air sacs, liver and other tissues of a captive New World toucanet (Reavill 1996).

Columbia livia excreta samples from several places in Bolívar state, Venezuela was evaluated by Cermeno *et al* (2006) for the presence of primary pathogen fungi. Filamentous fungi such as: *Aspergillus* sp. (31.1%), *Mucor* sp. (20.2%), *Penicillium* sp. (9.5%) and *Fusarium* sp. (6.7%) were the most frequently isolated strains. Species such as *Candida albicans* (4.1%), *Cryptococcus albidus* and *Rhodotorula* spp (2.7%), *C. neoformans var neoformans* (1.4%), *Trichosporum asahii* (1.4%), *Curvularia*, *Microsporum* and *Phoma* as well as *Histoplasma capsulatum* (1.3%) were less frecuently isolated. Kocan and Hasenclever (1972) isolated *Candida* sp. and *Geotrichum candidum* from the upper digestive tract. Ramirez *et al* (1976) isolated fourteen species of fungus from the lower digestive tract of 69 out of 80 pigeons. Fungi isolated were characterized as *Allescheria boydii, Aspergillus* sp., *Candida krusei, Chrysosporium* sp., *Geotrichum candidum, Mucor* sp., *Paeciliomyces* sp., *Penicilhum* sp., *Rhizopus* sp., *Rhodotorula* sp., *Scopulariopsis* sp., *Streptomyces* sp. and *Trichosporon cutaneum.*

The ecological relationships between birds and feather-degrading fungi are poorly studied, but they appear to be similar to those for birds and feather-degrading bacilli (Burtt and Ichida 1999). *Chrysosporium* was identified in the plumage of birds as keratinolytic fungi. Feathers, nails and beaks of one hundred and twenty common birds in Nigeria: chicken (50), ducks (20), turkeys (15) and pigeons (35), were examined by Efuntoye and Fashanu (2001) using the soil plate technique for their mycoflora. Fifteen species of fungi were recovered and they belong to the genera *Chrysosporium, Trichophyton, Microsporum, Aspergillus, Fusarium, Mucor, Rhizopus, Penicillium* and *Trichoderma. Microsporum gypseum* was the species most frequently isolated (35% of the samples). The most common genus was *Chrysosporium* and *C. keratinophilum* was the species with the highest frequency in the genus (28.3%).Two species of *Aspergillus* were recorded and these were *A. flavus* (19.2% of total) and *A. niger* (26.7% of total). The *Penicillium* sp. isolated were *P. chrysogenum* and *P. funiculosum* and they occurred in low frequencies (1.7%) in the birds examined. *Fusarium, Mucor, Rhizopus* and *Trichoderma* spp. were isolated in low frequencies and were represented in 0.8–6.7% of the total birds examined. Keratinophilic fungi were also isolated by Dixit and Kushwaha (1991) from feathers of most common Indian birds,*viz.* domestic chicken (*Gallus domesticus*), domestic pigeon (*Columba livia*), house sparrow (*Passer domesticus*), house crow (*Corvus splendens*), duck (*Anas* sp.), rose-ringed parakeet (*Psittacula krameri*). Out of 87 birds, 58 yielded 4 keratinophilic fungal genera representing 13 fungal species and one sterile mycelium.*Chrysosporium* species were isolated from most of the birds. *Chrysosporium lucknowense* and *Chrysosporium tropicum* were the most common fungal species associated with these Indian birds. Maximum occurrence of fungi (47%) was recorded on domestic chickens and the least number of keratinophilic fungi was isolated from the domestic pigeon and duck.

The prevalence of fungal flora in the tracheal epithelium of wild birds in a rehabilitation centre in Spain was studied by Garcia *et al* (2007). Two hundred and sixteen birds representing 26 species from seven orders were sampled. *Candida, Rhodotorula*, *Sacharomyces cerevisiae*, *Trichosporon, Geotrichum penicillatum*, *Aspergillus, Penicillium*, *Alternaria, Mucor*, *Nigrospora*, *Gliocadium*, *Chrysosporium* were isolated from 92 of the birds sampled (42.5%); in 24.5% only yeasts, in 12.5% only moulds and in 5.5% both moulds and yeasts together. The cattle egret was where the greatest number of animals with positive isolation was detected. Within the yeast flora, basically there were strains belonging to the genus *Candida*, mainly *C. albicans*. In the moulds, almost a half of them belonged to the genus *Aspergillus*.

Aspergilus, Rhizopus, Penicilium, Mucor and *Fusarium* species was isolated by Nyakundi and Mwangi (2011) from *Leptoptilos crumeniferus* (marabou stork) droppings in Kenya.

2.3.2 Yeasts

Cryptococcus neoformans is an opportunistic basidiomycete yeast that causes cryptococcosis, a life threatening infection that is usually manifested as meningeoencephalitis mainly in immunocompromised conditions such as in patients with AIDS, post organ transplant surgery or hematological malignancies (Casadevall and Perfect 1998, Rozenbaum and Goncalves 1994, Ruiz *et al* 1982) . Non- HIV-related cryptococcosis has also been reported including cutaneous cryptococcosis, pulmonary cryptococcosis and cryptococcal infection of the central nervous system (Yao *et al* 2005). This yeast has 5 serotypes (A, B, C, D, and AD), and recently was subdivided into 3 varieties known as *C. neoformans* var. *grubii* (serotype A), *C. neoformans* var. *neoformans* (serotype D), and *C. neoformans* var. *gattii* (serotype B and C) (Lengeler *et al* 2001). The most important source of *C. neoformans* var. *neoformans* throughout the world is pigeon excreta and soil contaminated with avian droppings (Swinne Desgain 1975, Schonheyder and Stenderup 1982). Neilsen *et a*l (2007) reported *C. neoformans* exhibited prolific mating on pigeon guano, whereas *C. gattii* did not. Thus, pigeon guano represent the realized ecological niche for *C. neoformans* and a fundamental but not a realized niche for *C. gattii*. Based on these studies, it was hypothesized that an ancestral *Cryptococcus* strain gained the ability to sexually reproduce in pigeon guano and then swept the globe. *Cryptococcus neoformans* var. *neoformans* has been isolated from different sources in nature. In several countries, avian droppings, especially pigeon droppings, have been considered to be the major environmental source of *C. neoformans* serotype A and D (Mahmoud 1999, Kielstein and Bocklish 2000, Yimtubezenash *et al* 2001). The reason for the high occurrence of *C. neoformans* in avian excreta is believed to be due to a utilization of nitrogenous compounds such as uric acid and creatinine in excreta (Lattman and Walter 1968). It has also been isolated from droppings of canary, budgerigar, parrot, pheasant, macow, munia birds and other avian species (Pal 1978, 1989), old pigeon excreta probably remains the chief reservoir and source of *C. neoformans* (Pal 1997). The pigeon is unlikely to be the main source of *C. neoformans* in nature because only low concentrations of organisms are found in samples from the beak, feet, crop, and rectal swabs. The internal temperature of the pigeon is 42 °C, which inhibits the multiplication of *C. neoformans*. The high concentration of ammonia in fresh droppings is also inhibitory to growth. In contrast, very high concentrations of the yeast form of the organism are found in weathered pigeon droppings, an environment that is unfavorable to the growth of most microorganisms. *Cryptococcus neoformans* remains viable on dry pigeon droppings for several years. This can be a reservoir of persisting small capsules that are compatible with alveolar deposition. Dry excrement is a favorable substratum since it has fewer bacteria and therefore less competition, which could help explain the higher population density found in this substratum (Ruiz *et al* 1981). In many situations, reports of cryptococcosis have been related to pigeon droppings as the source of infection. However, an epidemiological analysis revealed that patients with pigeon contact had a high exposure risk. (Litman and Borok 1968). The old accumulated pigeon excretas are appropriate source for the isolation of *C. neoformans* (Emmons 1995). It has been reported that *C. neoformans* was not found from fresh pigeon droppings and pigeon cloaca samples (Mishra *et al* 1981). Pal (2005) analyzed 45 parrot faeces in Gujarat for the prevalence of *C. neoformans*. The pathogen was demonstrated in nine samples giving a prevalence of 20%. Of these isolations, eight were obtained from the dry and old droppings. However, a solitary isolate of *C. neoformans* was recovered from the fresh droppings.

Three hundred and sixty five samples of avian droppings, collected from parks and zoo, were investigated for the occurrence of *C. neoformans* in Korea by Chee and Kim (2003). Thirteen samples were positive for *C. neoformans*. All isolates were obtained from withered pigeon droppings**.**

Cryptococcus neoformans environmental isolates was recovered by Zarrin *et al* (2010) from pigeon droppings in different urban areas in Ahwaz, Iran. Of the 65 samples, 22 (34%) were positive for *C. neoformans* and this study showed the presence of *C. neoformans* in urban environmental sources at places with a large population in Ahwaz.

Cryptococcus neoformans was recovered from 5 of the 18 (7 macows, 6 parakeets and 5 parrots) avian excreta of the psittacine birds giving a prevalence of 27.7 per cent (Pal *et al* 2004). The positive isolations came only from the parrot droppings. The pathogen could not be demonstrated in the faeces of 7 macows and 6 parakeets.

The presence of *C. neoformans* was studied by Caicedo *et al* (1999) in bird excreta and in the air circulating in and around bird cages in the City Zoo of Cali, Colombia, between August 1994 and April 1995, using a sunflower seed agar culture medium for fungus isolation. A total of 380 samples was studied, 110 from droppings and 270 from petri dishes placed inside (148) and outside (122) the cages. *Cryptococcus neoformans* var. *neoformans* was found in only two cases, one from bird excreta (0.9%) and the other from air inside a cage (0.7%). The former positive sample was collected from the cracks of a dead tree where two crested caracaras (*Polyborusplancus*) roosted; the feces were dry, accumulated and with a pH of 6.

It was found by Nawange *et al* (2011) that out of 50 excreta samples collected in Jabalpur (India), 37 samples (74%) were found positive for *C. neoformans*, and samples were collected from pigeon houses, which were located inside the residential places. The findings of the study revealed the growing diverse ecological niche of *C. neoformans* in Central India.

The samples of bird excreta from pigeons, parrots, open billed storks and crows obtained from thirteen places in Bangkok and nearby areas between April and July 2004 was evaluated by Soogarun *et al* (2006). *Cryptococcus neoformans* var. *neoformans* was recovered only in one sample of pigeon excreta (9.09%). *C. neoformans* var. *grubii* was isolated from 36 (25.53%) out of 141 Passerine and Psittacine excreta samples from captive birds in the state of Parana, Brazil (Lugarini *et al* 2008).

Nine hundred and eighty three specimens of pigeon droppings collected in different regions of northern Iran was examined by Khosravi (1997). Of these samples, 175 (17.8%) were positive for *C. neoformans*. All isolates obtained *were C. neoformans* var. *neoformans*. Most of these isolates of C. neoformans were from pigeon shelters. Saprophytic fungi were isolated from all samples. *Aspergillus, Candida, Mucor* and *Pencillium* spp. were the most frequently isolated saprophytes.

Ninety five isolates of the *neoformans* variety of *C. neoformans* were recovered from 550 samples of avian droppings(pigeon and sparrow) from Gharbia Governorate, Egypt by Mahmoud(1999).

. Twenty-eight samples of old and dry pigeon droppings collected from different sites in Kathmandu, Nepal were investigated for the presence of *C. neoformans* by Pal (1997) . The organism was isolated from seven (25%) of the specimens. Seventy-two pigeon dropping samples were collected from 26 different localities in Seoul (Korea) and investigated for the occurrence of *C. neoformans* by Chee and Lee (2005). Seventeen samples from 8 different localities were found to be positive for *C. neoformans*. All isolates were obtained from withered pigeon droppings. All isolates belonged to *C. neoformans* var. *grubbi* (serotype A).

The faecal samples from 59 species of captive birds kept in cages at a local zoological garden, belonging to 12 different orders were analyzed by Abegg *et al* (2006). Thirty-eight environmental isolates of *C. neoformans* were obtained only from Psittaciformes (Psittacidae, Cacatuidae and Psittacula). Thirty-three isolates (87%) were from the var. *grubii*, serotype A, and five (13%) were *C. gattii*, serotype B.

2.3.3 Bacteria

The members of the family *Enterobacteriaceae* including *Salmonella* species, *Escherichia coli*, *Enterobacter aerogenes* and *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus mirablis*, and *Providencia alcalifaciens* pathogenic to humans (Vilcins *et al* 2002).

Free-living pigeons are potential reservoirs for several pathogenic microorganisms, including *Chlamydophila psittaci* and bacteria belonging to the genus *Salmonella*. In Japan, *S. typhimurium* and *C. psittaci* have been isolated with a high frequency from feral pigeons (Casanovas *et al* 1995, Pasmans *et al* 2004). *Chlamydophila psittaci* DNA also has been detected in the faeces (16%) of feral pigeons in north-eastern Italian towns (Magnino *et al* 2009). *Chlamydophila psittaci* was detected by Tarsitano *et al (*2010) in two pigeon guano samples in an Urban Setting in the City of Bari (Apulia Region) Italy. *Salmonella* species were not found. Megraud (1987) isolated *Campylobacter jejuni* from 106 of 200 pigeon feces samples tested.

Salmonella was isolated in an incidence of (7.04%) from faecal samples in purebred pigeons by Methner and Lauterbach (2003). Dovc *et al*(2004) recorded that *Salmonella* spp. were isolated from (5.7%) of the cloacal swabs of free-living pigeons in the city of Ljubljana, Slovenia. Pasmans *et al* (2004) isolated (22.8%) of *S. Typhimurium var. Copenhagen* from pooled faecal samples from pigeon lofts from the city of Ghent (Belgium). Sonntag *et al* (2005) considered the pigeons as a possible reservoir of Shiga toxin 2f-producing *E. coli* associated with human disease. Lillehaug *et al* (2005) detected *Campylobacter jejuni* in fresh faecal samples of six out of 200 feral pigeons while all samples were negative for salmonellae. Tanaka *et al* (2005) isolated salmonellae from (3.9%) of faecal samples from feral pigeons in Japan. McCrea *et al* (2006) isolated *Campylobacter* and *Salmonella* spp. from live bird to prepackaged carcass for 3 flocks of squab. Pedersen *et al* (2006) detected *S. enterica* in an incidence of (3.2%) from pigeons captured in Fort Collins, Colorado.

Wild birds are common environmental reservoir of Salmonellae, but the incidence of the organism in wild birds in general tends to be very low (Kirk *et al* 2002, Reche *et al* 2003, Abulreesh *et al* 2007). Salmonella species are either completely absent or exhibit very low prevalence in pigeons (Cizek *et al* 1994, Casanovas *et al* 1995, Hubalek *et al* 1995, Kirk *et al*

2002, Refsum *et al* 2002, Reehe *et al* 2003, Vlahovic *et al* 2004, Dovc *et al* 2004, Tanaka *et al* 2005, Lillehaug *et al* 2005, Pedersen *et al* 2006, Kobayashi *et al* 2007).

400 fresh faecal samples of rock pigeons in Makkah city were analyzed by Abulreesh (2011), western Saudi Arabia and found 2.5% and 2% of samples to be positive for shiga-toxin producing *E.coli* 0157 and *Salmonella* respectively. Shiga –toxin producing *E. coli* and *Salmonella* species were found in faecal droppings and/or cloacal swabs of pigeons that live in urban and rural areas around the world (Morabita *et al* 2001, Haag-Wackernagel and Moch 2004, Kobayashi *et al* 2007, 2009, Wani *et al* 2004)

The prevalence rate (28.16%) of bacterial isolates of public health importance in pigeons was reported by Ibrahim (2007). The incidence of bacterial pathogens differed according to health status of examined pigeons and ages either squabs or adults, as it gave the higher incidence in freshly dead squabs (33.33%) and in adults (28.57%) followed by diseased squabs (31.03%) and adults (26.67%) then finally slaughtered pigeons (25.56%). There was a wide range of bacterial pathogens isolated from nasal and cloacal swabs of diseased pigeons including *Camylobacter jejuni, Citrobacter freundii, Diplococcus pneumoniae, E.coli, K. oxytoca, K. pneumoniae, Mannheimia haemolytica, P. aeruginosa, Salmonella* sp., *S. aureus*and *Y. enterocolitica.*

House crows (*Corvus splendens*) in Selangor, Malaysia were examined by Ganapathy *et al* (2007) for the presence of *Campylobacter* species and *Salmonella* species, by serology, culture and PCR. For the detection of *Campylobacter* and *Salmonella* species swabs were taken either from the intestine or cloaca. For campylobacter, 25·3 per cent of the crows were positive by culture, and the species identified were *Campylobacter jejuni* and *Campylobacter coli*. No *Salmonella* species were isolated. Yong *et a*l (2008) identified 9 different species of bacteria belonging to the family *Enterobacteriaceae* in fresh faecal samples of the large-billed crow (*Corvus* sp.) collected from the study site at Bangsar, an urban setting in Kuala Lumpur, Malaysia. They were *Citrobacter freundii*, *Enterobacter cloacae*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Kluyvera ascorbata*, *Salmonella arizonae*, *Salmonella typhi*, *Shigella flexneri* and *Shigella sonnei.*

The prevalence of six genera of bacteria from a sample of 387 cloacal swabs from 364 passerines and woodpeckers was determined by Brittingham *et al* (1988). The prevalence of bacteria were as follows: *Escherichia coli* (1%), *Pseudomonas* sp. (22%), *Salmonella* sp. (0%), *Staphylococcus* sp. (15%), *Streptococcus* sp. (18%), and *Yersinia* sp. (1%). The prevalence of *Streptococcus* spp. was higher in omnivorous species than in grainivorous species (20% versus 8%).

A field survey was conducted by Awad -Alla *et al* (2010) to evaluate the prevalence of bacterial infections among free-living white-ibis (*Nipponia nippon*) in which 92 bacterial isolates were recovered from 193 different internal organs of 55 apparently healthy ibis.

Escherichia coli and Salmonella sp. were isolated at the rates of 43.6% and 14.5% respectively. The other bacterial pathogens isolated were *Shigella* sp.(34.5%), *Enterobacter* sp. (21.8%), *Citrobacter* sp. (18.1%), *Kleibsella pneumoniae* (16.3%), *Stapphylococcus aureus* (10.9%) and *Proteus mirabilis* (7.2%).

According to Goodenough *and* Stallwood (2010) culturing microorganisms from blue tit (*Cyanistes caeruleus*) and great tit (*Parus major*) nests from the same study site demonstrated diverse microbial communities with 32 bacterial and 13 fungal species being isolated. Dominant bacteria were *Pseudomonas fluorescens, Pseudomonas putida,* and *Staphylococcus hyicus*. Also common in the nests were the keratinolytic bacteria *Pseudomonas stutzeri* and *Bacillus subtilis*. Dominant fungi were *Cladosporium herbarum* and *Epicoccum purpurascens. Aspergillus flavous, Microsporum gallinae,* and *Candida* were present in 30%, 25%, and 10% of nests, respectively. Although there were no differences in nest mass or materials, bacterial (but not fungal) loads were significantly higher in blue tit nests. Microbial species also differed interspecifically. As regards potential pathogens, the prevalence of *Enterobacter cloacae* was higher in blue tit nests, while *Pseudomonas aeruginosa* present in 30% of blue tit nests and was absent from great tit nests. The allergenic fungus *Cladosporium cladosporioides* was both more prevalent and abundant in great tit nests.

The isolation, characterization and identification of pathogenic bacteria *Bacillus, Enterobacter, Enterococcus, Proteus, Providencia, E. coli, Shigella, Salmonella, Citrobacter, Staphylococcus, Klebsiella, Morganella* was done by Nyakundi and Mwangi (2011) from *Leptoptilos crumeniferus* (marabou stork) droppings in Kenya .

2.4 Effects of heavy metals on birds

Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic (As), 5.7; cadmium (Cd), 8.65; iron (Fe), 7.9; lead (Pb), 11.34; and mercury (Hg), 13.546 (Lide 1992). These are stable (can not be metabolized by the body), but get bioaccumulated. Heavy metals are ubiquitous, highly persistent and non-biodegradable with long biological half-lives (Burger *et al* 2007). Compared to essential micro and macro-nutrient ions, heavy metals have more tendencies to accumulate in the tissues of animals (Azmat *et al* 2006). The 23 heavy metals are antimony(Sb), arsenic (As), bismuth (Bi), cadmium (Cd), cerium (Ce), chromium (Cr), cobalt (Co), copper (Cu), gallium (Ga) , gold (Au), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag), tellurium(Te), thallium (Tl), tin (Sn), uranium (U), vanadium(V) and zinc (Zn) (Glanze 1996). Among the heavy metals Fe, Cr, Cu, Ni, Zn and Mn have known essential functions in the living system. However, exposure of organisms to high concentration of these metals results in significant accumulation with toxic effects.

Most heavy metals have no natural biological functions in the body (e.g. Cd, Pb and Hg) and can be highly toxic and their toxic effects disrupt the existing biological balance. The route of contamination can be via inhalation, ingestion and skin adsorption. Once they enter the body, they accumulate in tissues faster than the body's detoxification pathways can dispose them and eventually a gradual build up of the chemicals will occur when released to the ecosystem through natural and anthropogenic routes (Beiglbock *et al* 2002, Fernades *et al* 2008). The main reasons for increasing concentration of metals in the environment include the use of inorganic fertilizers and pesticides. High levels of heavy metals in soil, water and animals have been reported in different parts of India due to pollution. Heavy metals from industrial waste contaminate drinking water, soil, fodder and food. The accumulation of such metals may block biochemical processes in the soil and facilitate the entry of toxic metals into food chains (He *et al* 2005).

Birds have been recognised as good integrators of environmental contamination because of their abundance, their wide distribution, their feeding at different trophic levels, and their long span of life (Rothschild and Duffy 2005). Heavy metal toxicities are of major concern in wild and captive avian species. In wild birds it is impossible to fully control heavy metal toxicity (Aizenberg *et al* 2006). Over the years among wildlife, birds have served as bioindicators for a number of environmental contaminants (Eens *et al* 1999, Gragnaniello *et al* 2001) particularly heavy metals (Mochizuki *et al* 2002) since they are visible, widely distributed in the ecosystem, sensitive to toxins and high on the food chain. Birds are generally exposed to heavy metals mainly by ingestion of food, drinking and by geophagy which is the practice of eating soil-like substances to obtain essential nutrients such as sulphur and phosphorous from the soil. Absorption rate varies among heavy metals depending upon intrinsic properties, species physiology and bioavailability in particular media. Heavy metals are important factors in the formation of free radicals which causes oxidative stress, inhibit repair of DNA damage and form adduct in nucleotide bases. Higher oxidative stress may cause high mutation rates in birds (Bickham *et al* 2000, Dahl *et al* 2001, Eeva *et al* 2006).

Once a metal has entered the body it can be stored or accumulated, or it can be excreted (Burger 1993). Birds can rid the body of heavy metals through the faeces or by depositing them in the uropygial gland, salt gland (Burger and Gochfeld 1985) and feathers (Burger 1993). Females can also eliminate heavy metals by sequestering them in their eggs, which may jeopardise the developing embryo. The faecal material as an indicator of metal pollution (consisting of unabsorbed and excreted absorbed metals) has shown to reflect the metal pollution level well in environment and food items, indicating especially the food chain contamination (Eeva and Lehikoinen 1996, Dauwe *et al* 2000, 2004). Feathers can be used as indicators of metal contamination because: (1) Birds sequester metals in their feathers, (2) the proportion of body burden that is in feathers is relatively constant for each metal, (3) a relatively high proportion of the body burden of certain metals is stored in the feathers (Burger 1993), and (4) there is a high correlation between levels of contaminants in the diet of seabirds and levels in their feathers (Burger 1993, Monteiro and Furness 1995). Breast feathers are the best indicator of whole-body burdens (Furness *et al* 1986).

The concentration and distribution of a given trace metal in the body depend on the intensity and duration of exposure, the form of the metals, interactions with other toxins, and a variety of factors intrinsic to the host (Gochfeld and Burger 1987). Chronic metal exposure to birds can result in detrimental effects on growth, development, reproduction, behaviour, resistance to diseases, and other physiological mechanisms (Heinz 1979, Grue *et al* 1986, Scheuhammer 1987, Snoeijs *et al* 2004, Dauwe *et al* 2005). Reproductive effects could include failure to build nests, decreased testis weight, spermatogenesis failure, decreased egg production, lighter eggs, eggshell thinning, increased embryo mortality, reduced hatching success, teratogenesis and lethargy, and behavioral abnormalities in chicks (Grandjean 1976, Heinz 1979, Scheuhammer 1987, Denneman and Douben 1993, Eeva *et al* 2000, Janssens *et al* 2003).

Heavy-metal-related impairments in birds are wide and diverse. Pb which often is present in relation to hunting activities, impairs the growth and survival of nestlings; causes haemolytic anaemia in wild Pb-poisoned birds; has adverse effects on reproduction, such as decreased plasma calcium and egg production; and causes behavioural impairments (Burger and Gochfeld 1994, Mateo *et al* 2003, Scheuhammer 1987). Franson (1996) categorized liver tissue lead levels of 2–6 µg/g to be subclinical exposure,3–6 µg/g to be toxic and 5 to 20 µg/g to be fatal in Galliformes, Falconiformes and Columbiformes respectively. Tsuji *et al* (2002) recorded a range of Pb levels ≥20 µg/g indicative of Pb toxicosis in wild mallard ducks. Cd is one of the most abundant nonessential metals present in the environment because of its use in industrial activities. This toxic metal is known to induce kidney toxicity, lesions in intestinal tissue, disruption of calcium metabolism, decreased food intake, and thinning of eggshells (Burger 2008, Hughes *et al* 2000, Mayack *et al* 1981). With continued exposure, even at low levels, this trace element is accumulated throughout the life span of birds (Dailey *et al* 2008). In birds, Hg concentrations of 0.5 µg/ g in egg and 9–20 µg/ g in feathers are correlated with decreased reproductive success in piscivorous species (Ochoa-acuna *et al* 2002). Hg also induces eggshell thinning and malformations; inhibits eggs production; and has embryotoxic effects (Heinz and Hoffman 2003, Lundholm 1995). This trace element is also associated with deleterious effects, such as decreased food intake, which leads to weight loss, weakness in wings and legs combined with coordination problems, and difficulties in flying and walking; paralysis, convulsions; and even death (Scheuhammer 1987). At high doses, essential elements, such as Cu, Zn and Se could also have toxic effects on kidneys and impair reproduction (Heinz *et al* 1989, Carpenter *et al* 2004).

2.4.1 Feathers

Birds can rid their bodies of heavy metals through both excretion and deposition in feathers, and females can also eliminate heavy metals in the contents of their eggs. The levels of Cd, Cr, Pb, Mn, Hg and Se were examined in four species of birds near Sydney, New South Wales, Australia in January, 1996 (Burger and Gochfeld 1999). Molted contour feathers were collected from silver gull, *Larus novaehollandiae* (Royal National Park and downtown Sydney), sulphur-crested cockatoo, *Cacatua sulphurea* (Blue Mountains, Royal National Park, and Sydney), Australian white ibis, *Threskiornis molucca* (Sydney), and rock dove, *Columba livia* (Royal National Park and Sydney). There were significant species differences in all metals, with rock doves having the highest levels of Cd, Cr, Pb and Mn, and silver gulls having the highest levels of Hg and Se. Metal levels were generally low in cockatoos, and were lowest in those from the Royal National Park. For silver gulls, Cd, Pb and Cr levels were highest at Sydney, and there were no locational differences in manganese, mercury, and selenium levels. For rock doves, Cd, Cr and Mn were higher in Sydney, and there were no locational differences in Pb, Hg and Se. Overall, Cd and Cr levels were significantly higher in Sydney than in the Royal National Park for all species, and there were no locational differences in Hg levels. Although the levels of most metals in feathers from these Australian birds were within the ranges reported worldwide, Pb levels in ibises and rock doves were among the highest reported worldwide.

The Pb exposure conditions using unwashed feathers of feral pigeons as a monitor for Pb pollution from rural, central urban, and four industrial complex areas in Korea with different ambient Pb concentrations was investigated by Nam *et al* (2004). Overall, the Pb levels in the feathers increased when the atmospheric Pb levels increased, so that the Pb levels in the feathers from urban and industrial areas were two to four times greater than those in the rural area.

The concentration of metals such as Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Ni, Pb and Zn were analyzed by Malik and Zeb (2009) in the feathers of cattle egret (*Bubulcus ibis*) from three breeding colonies in the Punjab province, Pakistan. The mean concentrations of Ca, Cd, Fe, Pb and Mn were significantly different between the three study sites (River Chenab, River Ravi and Rawal Lake Reservior). The mean concentrations of Ca, Cd, Fe and Mn were significantly greater at the River Chenab heronry and the Cr, Co, Zn and Pb concentrations at the River Ravi heronry. The feathers of cattle egrets collected from the Rawal Lake Reservior heronry were least contaminated. The study suggested that the feathers of cattle egret should be used as a biomonitor of the local heavy metal contamination.

The levels of As, Cd, Cr, Pb, Mn, Hg and Se in feathers from common eiders (*Somateria mollissima*), glaucous-winged gulls (*Larus glaucescens*), pigeon guillemots (*Cepphus columba*), tufted puffins (*Fratercula cirrhata*) and bald eagles (*Haliaeetus* *leucocephalus*) were examined from the Aleutian Chain of Alaska by Burger and Gochfeld (2009). There were significant interspecific differences in all metal levels. As predicted bald eagles had the highest levels of As, Cr, Pb and Mn, but puffins had the highest levels of Se, and pigeon guillemot had higher levels of Hg than eagles (although the differences were not significant). Common eiders, at the lowest trophic level had the lowest levels of some metals (Cr, Hg and Se). However, eiders had higher levels than all other species (except eagles) for As, Cd, Pb and Mn. Levels of Pb were higher in breast than in wing feathers of bald eagles. Except for Pb, there were no significant differences in metal levels in feathers of bald eagles nesting on Adak and Amchitka Island; Pb was higher on Adak than Amchitka. Eagle chicks tended to have lower levels of Mn than older eagles.

2.4.2 Egg and egg shells:

The levels of heavy metals (Pb, Cd, Hg, Se, Mn, Cr) was estimated in the contents and shells of eggs of roseate terns (*Sterna dougallii*) and herring gulls (*Larus argentatus*) nesting at Cedar Beach, Long Island by Burger (1994). For both species, metal concentrations were significantly higher in the contents compared to the shells for Pb, Hg, Se and Cr. For herring gulls, metal levels were higher in the shells for Cd and Mn. Levels of Cd, Hg and Se were significantly higher in roseate tern egg contents than for herring gulls. In eggshells, Pb, Cd, Hg and Se were significantly higher in roseate terns compared to herring gulls. For both species, eggshells account for about 7-8% of the egg by weight, but less than 1% of the egg burden for Hg, 1-5% for Pb, Se and Cr and 7-11% Mn. For Cd, shells account for only 5% of the egg burden for roseate terns, but 29% for herring gulls. These data suggest that, except for Hg, eggshells provide another method of excretion of metals in these two species of birds.

It has also been examined that eggs of the great tit (*Parus major*) could be used as indicators for Pb, As, Cd, Cu and Zn pollution (Dauwe *et al* 2002). Eggs collected from two sites with different pollution levels, and heavy metal levels were measured in the egg content and eggshell separately. At the polluted site, situated near a metallurgic factory in Hoboken (Belgium), eggshells contained significantly higher concentrations of As, Cd and Pb than did eggshells at the reference site on the campus of the University of Antwerp. Egg contents also contained significantly higher concentrations of Pb at the polluted site than at the reference site. Both at the polluted and reference site egg contents contained higher concentrations of Zn and Cu than did the eggshell. The eggshell contained higher As and Pb concentrations than did the egg content at the polluted site but not at the reference site. There was a clear discrepancy between essential and non-essential elements. Cu and Zn, two essential elements, were highest in the egg content, while As and Pb were higher in the eggshell. Moreover, the concentrations of essential elements did not differ significantly between the two sites. Eggs of blue tits (*Parus caeruleus*) also collected from the polluted site, and compared metal levels between the eggshell and egg content. Differences between the shell and content were similar to those for the great tit. At the polluted site no significant differences in metal levels were found between the two species. This indicated that great and blue tits sequester non-essential heavy metals in their eggs, especially in the eggshell. Therefore the eggshell is suitable as an indicator for heavy metal pollution. Burger (2002) examined the levels of seven metals in the eggs of five species of marine birds that nest in Barnegat Bay, New Jersey to determine whether there are differences among species and whether such differences reflect food chain differences. There were significant differences among species for all metals, except Cd with black skimmers (*Rynchops niger*) having the highest levels of all metals except Mn and Se. Metal concentrations in eggs mainly represented food chain differences. Hg exhibited the greatest interspecific difference, with skimmer eggs having five times higher Hg levels than the eggs of great black-backed gulls (*Larus marinus*). Although there were significant interspecific differences in the other metals, they were generally less than an order of magnitude. There were few high, significant correlations among metals, although Hg was positively correlated with As overall. Mean Hg levels exceeded the level known to adversely affect development in bird eggs for common (*Sterna hirundo*) and Forster's (*Sterna forsterii*) terns and for skimmers and exceeded the mean for eggs of fish-eating birds reported from 68 studies.

Cd, Cr and Pb concentrations were estimated in the eggshells of six species of the bird family *Ardeidae, viz., Bubulcus ibis, Egretta garzetta, Ardeola grayii, Ixobrychus minutus, I. cinnamomeus* and *Botaurus stellaris* were estimated from 13 different sites in the three Barak Valley districts of Cachar, Hailakandi and Karimganj of Assam by Dev *et al* (2010). While Cd levels were low and Cr levels within reasonable limits, Pb concentrations were relatively high.

2.4.3 Tissues

The concentrations of Pb, Cd and Mn were compared in the tissues of cattle egrets (*Bubulcus ibis*) and laughing gulls (*Larus atricilla*) gathered from the Galveston Bay region of Texas by Hulse *et al* (1980), to determine if different patterns of accumulation exist. Their levels in these species were within the range reported for other bird species. Pb levels in bone were comparable, but gulls had more Pb in brain, kidney and liver tissues than the egrets, which suggested a higher rate of accumulation or exposure. Due to their high abundance and comparable positions in the estuarine and terrestrial food webs, it is suggested that *Bubulcus ibis* and *Larus atricilla* may serve as convenient biological indicators to monitor potentially toxic substances in these ecosystems.

The Cd concentrations were determined in the blood and in different tissues of wild birds exposed to environmental Cd by Garca-Fernandez *et al* (1996). They studied a total of 118 birds living in the southeast of Spain, a zone with a Mediterranean climate and stopover point for important migratory species. The distribution pattern followed by the Cd in the samples reveals that the kidney is the primary organ for accumulation, followed by the liver and, to lesser extent, the brain and bone. Low concentrations were found in the tissues and in the blood. The study of correlations between the different tissues and blood suggests a compartmental behavior of Cd under these exposure conditions.

A study was conducted by Zaccaroni *et al* (2003) to determine Cd, Cr and Pb concentrations in liver and brain of 52 little owls (*Athene noctua*) from two provinces of Emilia Romagna region(Bologna and Parma, Italy). For all metals highest mean concentrations were found in liver (170 ppb for Cd, 297 ppb for Cr and 312 ppb for Pb).

The concentrations of heavy metals (Mn, Zn, Pb and Cd) were studied by Kim *et al* (2009) in tissues in six orders of Korean wild birds ($n = 37$), 2000–2002. Zn, Mn, Pb and Cd concentrations in all tissues were highest in ancient murrelets (*Synthliboramphus antiquus*). Essential elements in Korean wild birds were within the normal range for wild birds and are maintained there by a normal homeostatic mechanism. Pb concentrations in livers of almost all birds were within the background levels. Cd concentrations in livers and kidneys of ancient murrelets exceeded background levels for wild birds, but other birds were within the normal range. Acute and chronic contaminations of Pb and Cd levels differed among groups (or species). It was suggested that differences in Pb and Cd concentrations among groups are because of differences in diet and habitat. In ancient murrelets, Zn and Mn concentrations correlated with their Cd concentration in livers. Zn, Mn and Cd concentrations in murrelet livers were also higher than in other species.

The data on Pb, Cu, Zn, Se and As for bone and liver in marbled teal (*Marmaronetta angustirostris*) and white-headed duck (*Oxyura leucocephala*) was presented by Taggart *et al* (2009). For most metals, white-headed ducks had higher levels in liver and bone than marbled teal. The white-headed ducks showed higher levels of metals than marbled teals, female white-headed ducks commonly had significantly higher metal levels than males, and the reverse was true for marbled teal. In a review of metal levels in vertebrates (Burger 2007), 30 of 43 studies showed levels tended to be higher in females than in males, and in waterbirds. Taggart *et al* (2006) found higher As, Zn and Pb in livers from females than from males. In contrast, Braune *et al* (2005) found higher levels of Hg, Cu and Se in male long-tailed ducks (*Clangula hyemalis*) than in females and Warren *et al* (1990) determined the same trend for Cr, Ni, Pb and Cu in blue-winged teal (*Anas discors*).

Trace elements (Hg, Cd, Cu, Zn, Pb, Al, Ni, As and Se) were found in liver, kidney, muscle, and feather of aquatic birds wintering or inhabiting the wetlands situated on the Southwest Atlantic coast of France (Lucia *et al* 2010). A majority of greylag geese, red knots, and grey plovers were collected from among huntershot animals. The relation between residue concentrations, age (juvenile vs. adult), and sex was investigated. Trace elements were lower than threshold levels of toxicity, except for Pb. Greylag geese sampled could be considered Pb-poisoned. These consequential levels of contamination could be the result of the ingestion of Pb-shot from ammunition used in hunting areas they crossed during migration. Cd accumulation increased with age, whereas Pb levels in feathers were lower in adult birds in connection with moulting. The concentration of As was influenced by sex, female birds displayed higher concentrations in liver and feathers than did male birds.

Study had been carried out by Geta (2010) between July 2009 and June 2010 on the food chain components of Lake Ziway. Bioaccumulation and biotransference of heavy metals were measured and determined in tilapia (*Oreochromis niloticus*) and Abyssinian ground hornbill (*Bucorvus abyssinicus*) to evaluate ecological hazard levels. Heavy metals Cu, Zn, Cd and Pb were investigated with mean concentrations of 1.13 mg/kg, 27.13 mg/kg, 0.35 mg/kg and 0.28 mg/kg respectively in tilapia and 8.91 mg/kg, 139.08 mg/kg, 0.225 mg/kg and 0.286 mg/kg respectively in Abyssinian ground hornbill muscle tissue samples. Zn and Cu indicated biotransference up the food chain with 5 folds and 7.9 folds, respectively while Cd and Pb showed lower concentration in the predator bird. Thus, the detected heavy metals Zn and Cu indicated positive biotransference through the food chain.

The concentrations of selected 10 metals in 42 samples of house sparrow were compared by Albayrak and Mor (2011) in a polluted thermal power plant and reference sites in Turkey. The mean tissue concentrations of some metals to be significantly higher in sparrows from the polluted area when compared to tissues from the reference site. In the liver mean concentrations of Cu (35.85 ± 17.22 mg/ kg) and Zn (101.76 ± 26.38 mg /kg) were significantly higher and concentration of Ni (0.43 ± 0.49 mg /kg) were significantly lower in sparrows from the polluted area. The concentration of Cu was significantly higher in muscle and liver at the polluted site. Gender did not seem to influence residue levels, of the elements studied, among sparrows with the exception of kidney Co concentrations which were higher in female sparrows than in males.

The variation in metal contamination in six species of birds, namely the cormorant (*Phalacrocorax carbo*), cattle egret (*Bubulcus ibis*), little egret (*Egretta garzetta*), pond heron (*Ardeola grayii*), common myna (*Acridotheres tristis*) and jungle babbler (*Turdoides striatus*) in Nilgiris district, Tamil Nadu, India have been repoted by Jayakumar and Muralidharan (2011). The accumulation of heavy metals differed among the species studied. On an average, little egret accumulated high concentrations of Cu (53.31 ± 23.19 ppm) followed by cattle egret (16.27 ± 9.83 ppm) in liver. Of all the species, jungle babbler recorded the maximum concentrations (20.59 ± 9.07 ppm) in muscle. The pond heron recorded the maximum concentration (35.38 ± 11.14 ppm) in brain. On an average the maximum level was in the kidney of common myna (7.76 ± 1.80 ppm).

2.4.4 Excreta

The exposure of two hole-nesting passerines, the great tit (*Parus major*) and the pied fly-catcher (*Ficedula hypoleuca*), to heavy metals was studied by Eeva and Lehikoinen (1996) by measuring their faecal concentrations at 14 study sites around a Cu smelter complex in Harjavalta, south-west Finland in 1991–1993. Cu, Ni and Pb contents of nestling faeces were high near the factory and decreased with distance away from the pollution source. Dauwe *et al* (2000) concluded that excrement of great and blue tit nestlings can be used as a biomonitor for heavy metals (Pb, Cd, As, Cu), faeces metal concenteration, however, tells less about tissue concenterations (Berglund 2010).

The metal concentrations (Ag, As, Cd, Cu, Hg, Ni, Pb, Zn) was determined in the feathers and excreta of nestling great tits (*Parus major*), in their main invertebrate prey (Lepidoptera larvae) and in vegetation samples, all collected from four sites along a pollution gradient (Dauwe *et al* 2004). Metal contamination in vegetation samples increased significantly towards the pollution source. The Ag, As, Hg, Ni, Pb concentrations in food samples were significantly higher at the site closest to the pollution source compared to the other three sites. Great tit nestlings from the site closest to the pollution source had significantly higher concentrations of Ag, As, Hg and Pb in their excreta than did nestlings at the other three sites. For five metals (Ag, As, Cu, Ni and Pb), it was found concentrations in caterpillars to be significantly positively correlated with vegetation samples. A significant positive correlations between excreta and caterpillars for Ag, As, Hg and Pb and between feathers and caterpillars for As and Pb observed. Thus this suggest that excreta are a good monitor for the presence and concentrations of non-essential metals in the food and the environment of passerine birds.

Excreta and feathers of five species of adult passerine birds from Montepulciano wetland (Siena, Italy) was assayed for trace elements between January and August 2006 (Leonzio *et al* 2009). Pb concentrations varied from 16.31 to 26.50 mg/kg and were found strictly related to the age of feathers. Cu levels were found to be high mainly in insectivorous birds (9.68 mg/kg) and were probably influenced by local use of Cu-based agricultural fungicides.

The presence of heavy metals such as Zn and Cu in the Indian pond heron, little egret and cattle egret from northern Kerala have been reported by Seedikkoya and Shukkur (2004).

The concentrations of 11 trace metals in tissues from 10 body parts of great tits and greenfinches collected at Badachu Park in the Western Mountains of Beijing, China was examined to assess the metal accumulation level, distribution among body parts, and species and gender related variations (Deng *et al* 2007). The highest concentrations of Hg, Ni, Zn and Mn were found in the feather; Pb and Co in the bone; Cd, Cr and Se in the kidney and Cu in the liver and heart. Metal concentrations had substantial interspecific variation with great tits showing higher levels of Hg, Cr, Ni and Mn than greenfinches in tissues of most body parts. Gender related variations were body part and species specific. Gender related variation in trace metal accumulation could be related to ecological or physiological factors. Braune and Gaskin (1987) reported that female bonaparte's gull (*Larus philadelphia*) could excrete a higher percentage of the body burden of Hg than males in autumn molt. Moreover, there is gender related variation in the capability of eliminating trace metals. Females can sequester metals into eggs, which is an effective process for maintaining a lower body burden of trace metals than males (Burger and Gochfeld 1992, Dauwe *et al* 2002).

2.5 Effects of Pesticides on Birds

The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others. Ideally a pesticide must be lethal to the targeted pests, but not to non-target species, including man. Unfortunately, this is not the case, so the controversy of use and abuse of pesticides has surfaced (Aktar 2009). Birds are extremely mobile, and it is difficult to exclude them from areas that are treated with pesticides. Whether in agriculture where bird species attracted to agricultural insects pests as food can be economically important for the control of such pests (Kirk *et al* 1996) or in forestry, parks, or backyards, birds and pesticides interact. The most common route of exposure to pesticides is by ingestion of poisoned insects, carcasses, or grains intentionally treated with pesticides for bait (Hill and Fleming 1982, Quick 1982, Reece and Handson 1982). Pesticide induced death in birds is difficult to estimate accurately. Birds may die away from the site of poisoning or their carcasses decompose quickly or may be eaten by the scavengers. As a result, a small portion of such deaths are documented. For 187 globally threatened avian species, the primary pressure on survival is chemical pollution including pesticides. The mass mortality of the Indian Peafowl (*Pavo cristatus*), due to indiscriminate application of pesticides and herbicides in crop-fields are major causes of the recent decline in peafowl numbers (Ramesh and McGowan 2009).

About 5 million tons of pesticides are applied annually in the world, of which about 70% is used for agriculture and the remainder by public health and government agencies for vector control and by some owners (Yadav 2010). Three main groups of chemical synthetic pesticides are organochlorines(OCs), organophosphates (OPs) and carbamates (CMs). Some of the most troublesome pesticides are DDT (Dichlorodiphenyltrichloroethane), dieldrin, diazinon, parathion, aldicarb, atrazine, paraquat and glyphosate.

In India, 145 pesticides are registered for use at present. The production of pesticides started in India in 1952 with the establishment of a plant for the production of benzene hexachloride (BHC) near Calcutta, and India is now the second largest manufacturer of pesticides in Asia after China and ranks twelfth globally. There has been a steady growth in the production of technical grade pesticides in India, from 5,000 metric tons in 1958 to

102,240 metric tons in 1998. In 1996-97 the demand for pesticides in terms of value was estimated to be around Rs. 22 billion (USD 0.5 billion), which is about 2% of the total world market. The pattern of pesticide usage in India is different from that for the world in general. In India 76% of the pesticide used is insecticide, as against 44% globally (Mathur 1999). The use of herbicides and fungicides is correspondingly less heavy. During the DDT era about 85% of the farmers of the India used organochlorine pesticide at the rate of 0.39 kg/ha covering 282 million hectares of agricultural land (NCAER 1967). Now, the consumption of chemical pesticides is highest in Andhrapradesh (33%), followed by Punjab, Karnataka, Tamilnadu, Maharastra, Haryana, Gujrat, Uttar Pradesh and the remaining states account less than 9.5 percent of the total (Singhal 1969). Nearly 70% of the pesticides consumed in India are reported to be utilized for cotton (45%) and rice (22%) and such amount of pesticide use has remained almost unchanged during the last five decades (Vyas 1998). In India, pesticide use has increased dramatically and now it is becoming a global problem (US EPA 1978).

Pesticides/pesticides formulations banned in India are aldrin, BHC, calcium cyanide, chlordane, copper acetarsenite, bromochloropropane, endrin, ethyl mercury chloride, ethyl parathion, heptachlor, menazone, nitrofen, paraquat dimethylsulphate, pentachloronitrobenzene, pentachlorophenol, phenylmercury acetate, sodium methane arsenate, tetradfon, toxafen, aldicarb, chlorobenzilate, dieldrine, maleic hydrazidfe, ethyl dibromide TCA.

According to Pimentel (2001), 72 million birds die each year in US as a result of pesticide . In USA, some 50 pesticides have killed songbirds, gamebirds, raptors, seabirds and shorebirds (BLI 2004). ln a small area of the Argentine pampas, monocrotophos, an OP, has killed 6,000 Swainson's hawks. Worldwide, over 100,000 bird deaths caused by this chemical have been documented (Hooper 2002).

In UK, the volume of seeds eaten by many bird species is large enough to pose a potential risk if the seeds are treated with one of the more toxic fungicides (Prosser and Hart 2005). OP insecticides, including disulfoton, fenthion, and parathion are highly toxic to birds. These have frequently poisonined raptors foraging in fields (Mineau *et al* 1999). The number of birds found killed by pesticides, in the UK was at least 60 in 2006, and 55 in 2007. The affected species included buzzard, red kite, raven, crow, peregrine falcon, golden eagle, gull, barn owl, tawny owl, magpie, pheasant, rook, marsh harrier, dove, jackdaw, and chaffinch (PSD and Defra 2007, ACP 2008).

The following pesticides were identified as a cause of fatal bird poisoning: (1)CMs (aldicarb, bendiocarb, carbofuran), (2) OPs (chlorpyrifos, diazinon, isofenphos, malathion, mevinphos, phorate), (3) anticoagulant rodenticides (bromadiolone, brodifacoum, difenacoum), and alphachloralose (Isenring 2010)).

2.5.1 Organochlorines (OCs)

OCs are divided into three groups, *viz.* (1) the DDT related compounds, (2) the cyclodiene insecticides (aldrin, dieldrin, endrin, heptachlor and endosulfan) and (3) isomers of HCH. The acute toxicity of p, p'-DDT is attributed mainly to action on axonal voltage dependent Na^+ channels (Eldefrawi and Eldefrawi 1990). Normally when Na^+ current is generated during the passage of a nerve action potential, the signal is rapidly ended by the closure of the sodium channel. In DDT poisoned nerves, the closure of the channel is delayed causing disruption of action potential regulation which can lead to repetitive discharges (Walker 2001). Apart from the action on Na^+ channels, DDT or its metabolites also act as inhibitors of Ca^{++} ATPases in the membrane of avian shell gland cells and reduces the transport of $CaCO_3$ from blood into egg shell. This results in a dose dependent thickness reduction (Lundholm 1997). Populations of sparrowhawks (*Accipiter nisus)* and other birds of prey had declined and the cause was found to be a decrease in the thickness of their eggshells (causing eggs to break during incubation) and direct mortality from ingesting OCs (Newton 1995). DDE is responsible for the severe eggshell thinning of American kestrel, peregrine falcon, sparrow hawks and gannets (Wiemeyer and Porter 1970). Extensive ecotoxicological investigation on the effect of DDT on eggshell thinning in the Himalayan greyheaded fishing eagle was carried out (Naoroji 1997) and it was found that DDE residues found in eggs of affected bird were nearly 10 ppm (Peakall 1993). DDT has also caused local mass death of birds. LD_{50} of DDT in birds is<500 mg/kg (Edson *et al* 1966).The LD_{50} of dieldrin is 67mg/kg in pigeon (WHO 1989). Residues of dieldrin, heptachlor epoxide and other OCs in the tissues of British sparrow hawk and kestrel from 1963 to the 1990s are recorded (Newton and Wyllie 1992). The species show sharp declines in agricultural areas during the said period. Because of its lipophilicity and refractory character, the toxic effect of dieldrin may be carried out to the next generation (Moriarty 1968).

Cyclodienes primarily act as inhibitor of GABA receptor and reduce the flow of chloride ions (Krieger 2001) which leads to neurological disorders like tonic convulsions and clenched claws in predatory birds (Walker 2003).The cyclodiene compound, endosulfan alters the electrophysiological and associated enzymatic properties of nerve cell membranes and interferes in the kinetics of Na^+ and K^+ ion flow through the membrane (Hayes and Laws 1991). Endosulfan remains deposited in the adipose tissue and in stressed conditions like migration, breeding, illness or inclement weather, their fat is metabolized releasing endosulfan and caused adverse effects even after single exposure (Douthwaite 1995).

Endosulfan, a neurotoxic pesticide, is highly to moderately toxic to bird species. The cyclodiene endosulfan is highly toxic to birds (Kidd and James 1991). Administration of endosulfan by the dietary route resulted in lethargy, weakness and diarrhea in Japanese quail (Prakash *et al* 2009). Acute oral studies conducted in 3-4 months old mallards treated with endosulfan resulted in birds exhibiting wings crossed high over their back, tremors, falling and other symptoms after ten minutes of oral gavage dose administration. The diarrhea and the nervous symptoms produced by endosulfan are due to stimulation of the central nervous system (Hudson *et al* 1984). It is transported over long distances through the air and has been found in the Arctic far from any sources of use (Sang *et al* 1999).

Chronic low level OC exposure affects the reproductive success of birds and changes their mating behavior. Fry (1995) identified OC pesticides, industrial pollutants, OP pesticides, petroleum hydrocarbons, heavy metals, herbicides, and fungicides. p,p'-DDT, polychlorinated biphenyls (PCBs), and mixtures of OCs as environmental estrogens affecting populations of gulls breeding in polluted "hot spots" in southern California, the Great Lakes, and Puget Sound. Estrogenic OCs represent an important class of toxicants to birds because differentiation of the avian reproductive system is estrogen dependent. The United States Environmental Protection Agency (US EPA 2001) has identified endosulfan as a potential endocrine disrupter. Birds may be exposed to pesticides through contaminated seed consumption. Small birds are particularly at risk due to their low body weight. The birds face high risk due to the consumption of high quantities of seed (US EPA 2006). Lindane affects serum hormone level which is important in reproduction and metabolism. In ewes, concentrations of estradiol and insulin were significantly increased after administration of lindane, while concentrations of basal luteinizing hormone and thyroid levels decreased (Rawlings *et al* 1998). The reduced hormone levels resulted in decreased egg production (Herbst and van Esch 1991).

When fed with DDE for longer duration, courtship behavior in ring doves (Haegele and Hudson 1977) and nocturnal activity in white-throated sparrow (Mahoney 1975) were disturbed. Sub lethal doses of dieldrin affect the aggressive behavior of mallard duck, social and breeding behavior of bobwhite quail and a variety of effects in the pheasant (Peakall 1985).

The developing chicks showed malformed beaks and skeleton, fluid retention in their heart and problems in sex determination, after chronic sub lethal OC exposure (Gilbertson and Fox 1977). Congenital abnormalities and defects of feather growth of young terns are reported after OC exposure along the east coast of the USA (Bourne *et al* 1977).

Anaemia and decreased haemoglobin concentration have been documented after birds were exposed to lindane (Mandal *et al* 1986). Suppression of T-cell mediated immunity in the wild Caspian terns and herring gulls were found to be associated with high perinatal exposure to OC compounds (Grasman *et al* 1996). After administration of 2 ppm endosulfan in chicks for 8 weeks, there was a significant decrease in the number of T and B lymphocytes and total leucocytes along with atrophy and decrease in size of the follicles and hemorrhages in the thymus (Garg *et al 2004*).

The residues of BHC and DDT have been estimated by Kaphalia *et al* (1981) by gas-liquid chromatographic analysis of the internal body organs, depot fat, and blood plasma of a few species of Indian wild birds, captured in and around the urban area of Lucknow. Total BHC and gamma-BHC (lindane) levels were high in breast muscle, liver, heart, and lung tissues of pigeon, crow, and vulture, compared with the respective tissues of chicken, cattle egret, and kite. More lindane and total BHC was found in tissues of vulture compared with other species. The major part of BHC isomers in the brain of birds examined was accounted for by alpha-BHC. Total BHC detected in depot fat of crows was 29.7 ppm; lesser amounts were found in vulture, kite, and cattle egret, respectively. Total DDT levels were comparable in the blood plasma of chicken, pigeon, crow, and cattle egret, although residues generally showed the following order in the tissues examined: chicken less than pigeon less than cattle egret less than crow less than kite less than vulture. High levels of DDT were detected in depot fat of crow, kite, and vulture (50.8, 67.0 and 95.3 ppm respectively). Avian species thus reflect biomagnification of BHC and DDT residues, presumably due to their food habits. Walker *et al* (1967) reported OC insecticide residues in a wide range of wild birds and their eggs from all over Britain. Higher residues were found in predatory species than in omnivorous or herbivorous species.

The concentrations of OC pesticides were measured by Dhananjayan and Muralidharan (2010) in blood plasma of 13 species of birds collected from Ahmedabad, India. Among the various OCs determined, HCHs and its isomers had higher contribution to the total OCs. Concentration of ∑HCHs varied from 11.4 ng/mL in White ibis (*Threskiornis melanocephalus*) to 286 ng/mL in sarus crane (*Grus antigone*), while ∑DDT ranged between 19 ng/mL in black ibis (*Pseudibis papillosa*) and 147 ng/mL in painted stork (*Mycteria leucocephala*). *p,p′*-DDE was accounted for more than 50% of total DDT in many of the samples analysed. However, a *p,p′*-DDT to *p,p′*-DDE ratio higher than one obtained for many species of birds indicates the recent use of DDT in this study region. The concentrations of cyclodiene insecticides, heptachlor epoxide, dieldrin and total endosulfan ranged from 15.8 to 296.2 ng/mL, below detectable level to 15 and 41.1-153.2 ng/ml, respectively. The pattern of total OC load generally occurred in the following order: grainivores < insectivores < omnivores < piscivores < carnivores. Although, the organochlorine residues detected in blood plasma of birds are not indicative of toxicity, the presence of residues in birds over the years (2005-2007) indicates continued exposure to organochlorine compounds. However, continuous monitoring is recommended to facilitate the early identification of risks to the survival of a species.

OC pesticide residues were determined (Muralidharan *et al* 2008) in tissues of five Indian white-backed vultures and two of their eggs collected from different locations in India [carcasses from one each from Delhi, Patiala (Punjab), and Mudumalai Wildlife Sanctuary

(Tamil Nadu) and two from Ahmedabad (Gujarat) and two eggs from the vicinity of Bharatpur, Rajasthan during December 1999]. All the samples had varying levels of residues. p,p' DDE ranged between 0.002 µg/g in muscle of vulture from Mudumali and 7.30 µg/g in liver of vulture from Delhi. Relatively higher levels of p,p'-DDT and its metabolites were documented in the bird from Delhi than other places. Dieldrin was 0.003 and 0.015 µg/g while p,p'-DDE was 2.46 and 3.26 µg/g in egg one and two respectively. Dieldrin appeared to be lower than the threshold level of 0.5 µg/g. p,p'-DDE exceeded the levels reported to have created toxic effects in eggs of other wild birds. Although varying levels of DDT, HCH, dieldrin, heptachlor epoxide and endosulfan residues were detected in the vulture tissues, they do not appear to be responsible for the present status of population in India.

The residue levels of persistent OCs, such as HCH, DDT compounds, PCBs and HCB were measured by Ramesh *et al* (1992) in wildlife around the agricultural watershed of Parangipettai, South India. On the basis of overall concentrations HCH ranked first followed by DDT, PCB and HCB, reflecting the increasing usage of HCH in recent years in India. The residue levels of organochlorines in birds varied according to their feeding habits and showed the following pattern: inland piscivores and scavengers > coastal piscivores > insectivores > omnivores > granivores. High levels of HCH and DDT residues were recorded in pond heron and cattle egret which feed in the agricultural fields.

The residue levels of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and other organochlorines were analysed by Watanabe *et al* (2005) in the breast muscle of crows from a dumping site in the south of Chennai city, South India. Crows from the dumping site contained significantly higher total Toxic Equivalents (TEQs) (60 ± 27 pg/g lipid wt) than those from the reference sites (26 ± 18 pg/g lipid wt). Especially, certain dioxin-like coplanar PCB congeners (Co-PCBs), such as CB-77 and CB-105, whose source is commercial PCBs, were significantly higher in crows from the dumping site than those from the reference sites. Dioxin-like congeners in the soil of the dumping site were transferred directly to the crows through the ingestion of on-site garbage contaminated with soil.

Among the countries that continue to use OCs, India has been one of the major producers and consumers in recent years. As a consequence, wild birds in India are exposed to great amounts of OC pesticides (Tanabe *et al* 1998). Use of OCs in tropical countries may not only result in exposure of resident birds but also of migratory birds when they visit tropical regions in winter. The Indian sub-continent is a host to a multitude of birds from western Asia, Europe and Arctic Russia in winter (Woodcock 1980). Hundreds of species of waterfowl, including wading birds such as plovers, terns and sandpipers, migrate each winter to India covering long distances (Grewal 1990). Tanabe *et al* (1998) determined persistent OCs such as DDT and its metabolites, HCHs isomers, HCB and PCBs in whole-body homogenates of resident and migratory birds collected from South India. OC contamination pattern in birds varied depending on their migratory behaviour. Resident birds contained relatively greater concentrations of HCHs (14-8,800 ng/g wet wt) than DDTs and PCBs concentrations. In contrast, migrants exhibited elevated concentrations of PCBs (20-4,400 ng/g wet weight). The sex differences in concentrations and burdens of OCs in birds were pronounced, with females containing lower levels than males. Inland piscivores and scavengers accumulated greater concentrations of HCHs and DDTs while coastal piscivores contained comparable or greater amounts of PCBs. Global comparison of organochlorine concentrations indicated that resident birds in India had the highest residues of HCHs and moderate to high residues of DDTs. It is, therefore, proposed that migratory birds wintering in India acquire considerable amounts of HCHs and DDTs. Estimates of hazards associated with organochlorine levels in resident and migratory birds in India suggested that pond heron, little ringed plover, and terek sandpiper may be at risk from exposure to DDTs.

2.5.2 Organophosphates (OPs) and Carbamates (CMs):

OPs and CMs are most commonly used pesticides throughout the world because of their low bioaccumulation properties in comparison to OCs. Since the early 1980s, both OPs and CMs have been used as pesticide. Both these insecticides inhibit acetylcholinesterase (AChE) at the postsynaptic membrane of cholinergic synapses (Bishop *et al* 1998) in the central and peripheral nervous systems of all vertebrate species. OPs inhibit AChE by forming a phosphorylated enzyme derivative, making it more resistant to hydrolysis than the normal acetylated derivative (Taylor 1990). Inhibition of AChE leads to accumulation of the neurotransmitter acetylcholine at the synaptic cleft in the sympathetic and parasympathetic nervous system and in neuromuscular junctions, thus disrupting transmission across cholinergic synapses (Pope *et al* 1995). Irreversible inhibition of AChE results in continuous transmission and leads to seizures, respiratory failure and eventually death at high doses (Marrs 1996). Birds appear to be more sensitive to acute exposure to anticholinesterase pesticides due to a reduced level of anticholinesterase detoxifying enzymes (Parker and Goldstein 2000). Due to high activity of AChE in the brain of birds (Westlake *et al* 1983), the rate of binding to OP and CM is more rapid than other vertebrates (Hill 1992). Most OPs being the potent inhibitors, directly or indirectly (through the toxic metabolic byproduct) inhibit AChE (Extoxnet 1994). Other alternative sites of phosphorylation and direct effects of OPs on signal transduction pathways are known (Richards *et al* 1999), such as the inhibition of fatty acid amide hydrolase which affects limb immobility in OP-induced neuropathy (OPIDN) (Quistad *et al* 2001). OPIDN is characterized by the demyelination of nerve fibers and paralysis which were observed 2-3 weeks after single or repeated exposure (Grue *et al* 1997). Chloropyriphos, an OP, inhibits AChE in a way that has cross-generational implications (Anway *et al* 2005).

Inhibition of AChE by CMs either causes death within 30 min or is reversible with decarbamylation. While recovery from CMs usually occurs within 1-2 h, acute OP exposure causes avian mortality within 24 h (Hill 1992). The metabolism of latent inhibitors in the brain, amount and frequency of exposure and the sensitivity of brain AChE to inhibition are three most important causes of OP toxicity (Hill 1992). Mortality following exposure appears to be more often related to habitat preferences, physiological condition and /or foraging behavior than a species' ability to deal with actual toxic exposure (Mineau 1991).

Many CMs are reversible inhibitors and many organophosphorus pesticides are non-reversible inhibitors (Martin *et al* 1981), Cholinesterase(ChE) depression or inhibition followed by thermal reactivation can be employed to discriminate between OP and CM poisonings (Stansley 1993). Smith *et al* (1995) identified and confirmed pesticides in 86 of 102 incidents of mortality from 29 states within the USA from 1986 through 1991. Thermal reactivation of ChE activity was used to correctly predict CMs in 22 incidents and OPs in 59 incidents.

Parathion (Phosphorothioc acid O,O-diethyl 0-[4nitrophenyl] ester), an OP insecticide that has been widely used for many years, is highly toxic to birds. The median lethal oral dose (LD_{50}) of parathion varies from 0.125-0.250 mg/kg for fulvous whistling- ducks (*Dendrocygna bicolor*) to 24.0 mg/kg for chukar (*Alectorisc hukur*) (Schafer 1972). Accidental deaths of numerous wild birds have resulted from the agricultural use of parathion. White *et al* (1982a, b) reported the deaths of over 1,600 waterfowl, mostly canada geese (*Branta canadensis*), from applications of parathion to agricultural fields in Texas. Carson (1962) described the purposeful killing, by farmers, of an estimated 65,000 birds with parathion. Stone *et al* (1984) also reported two cases from New York. In the first, at least 5,120 birds, mostly red-winged blackbirds (*Agelaius phoeniceus*), common grackles (*Quisculus quisculu*) and brownheaded cowbirds (*Molothrus ater*) were killed by parathion treated corn. In the second, at least 3,196 birds, mostly common grackles, red-winged blackbirds and european starlings (*Sturnus vulgaris*), died after ingesting parathion- treated rye seed spread near unharvested field corn. A Cooper’s hawk (*Accipiter cooperii*) two red-tailed hawks (*Buteo jamaicensis*) and an American kestrel (*Falco sparverius*) were killed in these cases after consuming poisoned icterids. Small numbers of birds in six other species were also killed in these incidents. The two cases illustrate the ease with which grainivorous birds can be purposely killed with grain heavily treated with compounds such as parathion. Predators and scavengers may be secondarily poisoned after ingesting alimentary canals of poisoned birds.

The U.S. Department of Interior's National Wildlife Health Center reported that 50% of the documented cases of lethal poisoning of birds are caused by OPs and CMs (Madison 1993). The possible route of exposure of these pesticides is the consumption of seeds or insects contaminated on their surface with lethal amounts of insecticide (Prosser and Hart 2005). OPs have been implicated in 335 separate mortality events causing the deaths of about 9,000 birds in the US between 1980 and 2000 (Fleischli *et al* 2004). Worldwide, over 100,000 bird deaths caused by monocrotophos, a worst organophosphate, are documented (Hooper 2002). Application of diazinon, another widely used OP pesticide, to lawns, golf courses and turf farms have killed thousands of birds in U.S (Tattersall 1991). Diazinon predominantly affects herbivorous waterfowls like ducks and geese. Carbofuran, a CM pesticide alone is responsible for most bird deaths in California followed by diazinon (US EPA 1999). Analysis of brain AChE activity revealed that many birds have recovered from illness after sub lethal exposure to insecticides than die (Grue *et al* 1991). Although several reports are available on the short-term changes in behavior of birds, after exposure to sub lethal doses (Grue *et al* 1997) reports on the long-term changes in the behavior of birds appear to be few (Grue *et al* 1991).

Many OPs are acutely toxic to birds at very low doses. A recent compilation of acute lethal doses (LD_{50}s) for the mallard duck showed that 16 of 20 OPs were acutely toxic at doses less than 20 milligrams insecticides have a mode of action similar to the OPs and, like the OPs, some kill birds at low doses. Carbofuran, which has been estimated to kill one to two million birds annually in the U.S. (US EPA 1989) is probably the best known example. Granular formulations, in which the pesticide is incorporated with a carrier into small particles slightly larger than those found in granulated sugar (Meister 1991) have been particularly hazardous to birds. Over half of house sparrows fed granular formulations of three CMs (aldicarb, carbofuran, and bendiocarb) were killed by ingestion of just one granule and only five to ten granules killed red-winged blackbirds (Balcomb *et al* 1984). Mineau *et al* (2005) reported use of granular carbofuran resulted in an estimated annual mortality rate of 3.0 to 16 songbirds per ha of treated cornfield , the higher number corresponding to better field edge habitat. Much larger killings also occurred where fields bordered non-crop habitat more suitable for birds. At the peak of its popularity in the United States, this single product was conservatively giving rise to an estimated annual loss of 17- 91 million birds in cornfields alone. Another CM, carbaryl (Sevin), has an acute toxicity to birds several orders of magnitude lower (about 2000 mg/kg) (US EPA 1984) However, a study of effects on bird populations following a forestry application found that both the abundance of birds and the number of bird species present was reduced by aerial spraying of carbaryl, and these effects persisted until the summer following the spray. Although other studies of forestry carbaryl applications found no significant effects on birds, reviewers noted that this study lasted longer and used larger spray blocks than the studies that found no effects (Peakall and Bart 1983).

The U.S. Geological Survey National Wildlife Health Center (NWHC) mortality database from 1980 to 2000 was reviewed by Fleischli *et al* (2004) to identify cases of poisoning caused by OP and CM pesticides. From the 35,022 cases from which one or more avian carcasses were submitted to the NWHC for necropsy, 335 mortality events attributed to AChE poisoning which occurred in 42 states. Washington, Virginia, and Ohio had the highest frequency of events, with 24 (7.2%), 21 (6.3%), and 20 (6.0%) events, respectively. A total of 8877 carcasses of 103 avian species in 12 orders was recovered. Of 24 different pesticides identified, the most frequent were famphur ($n = 59$; 18%), carbofuran ($n = 52$; 15%), diazinon ($n = 40$; 12%), and fenthion ($n = 17$; 5.1%). Falconiformes were reported killed most frequently (49% of all die-offs) but Anseriformes were found dead in the greatest numbers (64% of 8877 found dead). The majority of birds reported killed by famphur were Passeriformes and Falconiformes, with the latter found dead in 90% of famphur-related poisoning events. Carbofuran and famphur were involved in mortality of the greatest variety of species (45 and 33, respectively). Most of the mortality events caused by diazinon involved waterfowl.

The pesticide poisoning of wild birds diagnosed at the National Veterinary Research and Quarantine Service (Kyunggi, Korea) from 1998 to 2002 was reported by Kwon *et al* (2004). Forty-one mortality events (759 birds) of 87 incidents (2,464 birds) investigated were associated with pesticide poisoning, and six OPs or CMs were identified as being responsible for the poisoning. Phosphamidon was most frequently identified as the cause of poisoning, accounting for 23 mortality events. Other pesticides identified as poisons for birds were OPs; monocrotophos, fenthion, parathion, Ethyl p-nitrophenyl (EPN), and diazinon, and the CM carbofuran. More than 10 to 1,000 times the minimum toxic dose of pesticide was commonly detected in the stomach contents of wild birds. There were 17 incidents involving waterfowl, such as mallards (*Anas platyrhynchos*), mandrin duck (*Aix galericulata*), and white-fronted goose (*Anser albifrons*), which were most frequently affected by pesticide poisoning. Other species affected in Korea included tawny owl (*Strix aluco*), cranes (*Grus monachus, Grus japonensis,* and *Grus vipio*), hill pigeon (*Columba repestris*) and oriental stork (*Ciconia boyciana*).

OPs and CMs intoxication are often associated with anorexia and symptoms of gastrointestinal stress (Grue *et al* 1991). Long-term effects of very small amount of OP affect the feeding behavior of breeding red-winged blackbirds (Nicolaus and Lee 1999). Exposure to OPs and CMs interferes with the bird's ability to discriminate between contaminated and clean foods. Reduction in body weight following sub lethal exposure with an average weight loss of 14% was also noted. Such weight loss is correlated with 55-77% AChE inhibition in European starlings after a single dose of dicrotophos (Grue and Shipley 1984). Lesions in lateral hypothalamus due to pesticide exposure lead to food avoidance and cause a sharp reduction in body weight in birds (Kuenzel 1994).

Alteration in the reproductive behaviour and gonadal development in birds (Kuenzel 1994) have been noticed following acute sub lethal exposure to OPs and CMs due to ventromedial hypothalamic lesions. Delayed development and degeneration of spermatogenic cells has occurred when domestic and semi-domestic birds were exposed to OPs. The decreased level of cholinesterase activity in testes and brains of adult male white-throated munia (*Lonchura malabarica*) is directly related to the increased number of degenerated germ cells in the seminiferous tubules, after exposure to methyl parathion (Maitra and Sarkar 1996). In another experiment, exposure of adult male rose-ringed parakeets (*Psittacula krameri*) to the graded doses of methyl parathion resulted in impaired testicular function which may be due to an altered circulating milieu of LH and testosterone (Maitra and Mitra 2008). When treated with two OPs such as methyl parathion and phosphamidon separately, the phosphamidon showed more potential effect and impaired gonadal functions even at very low sublethal doses in female spotted munia (Mitra and Maitra 2004). The most disturbing sub lethal effect that can be linked to OPs and CMs is their affect on the endocrine system causing reproductive and developmental damage within offspring. OPs insecticides impaired reproductive function possibly by altering secretion of luteinizing hormone and progesterone (Rattner *et al* 1984).

Alteration in the migratory behavior (Vyas *et al* 1995), sexual behavior (Grue and Shipley 1981, Hart 1993), litter and clutch size (Bennett *et al* 1991) and parental care (Grue 1982), are due to reduced levels of reproductive hormones which results from pesticide exposure. Reduction in singing and displaying in European starling (Hart 1993) and increased aggression in both sexes (Grue *et al* 1991) are strongly correlated with brain AChE inhibition. In OP exposed mallards, hatching success was reduced by 43% in comparison to controls due to abnormal incubation behavior including nest abandonment and extended time off nests (Bennett *et al* 1991). OPs and CMs reduce egg laying capacity. Reduction in food consumption alone is accounted for reductions in egg laying in Northern bobwhites fed a diet contaminated with methamidophos for 15 days (Stromborg 1986).

Alteration in the reproductive behaviour following ingestion of very-low concentrations of OP compounds may be endocrinological or pharmacological in origin. Studies suggest that OPs may influence reproductive functions in different vertebrates by reducing the brain AChE activity and monoamine levels and thus impairing hypothalamic or pituitary regulation on reproduction (Muller *et al* 1977). In female bobwhite quail, significant decrease in plasma titers of LH, progesterone and corticosterone (Rattner *et al* 1982) were noted following the short term ingestion of parathion. A variety of pharmacological agents that modify neurotransmitter levels act at the level of hypothalamus to adversely affect the reproductive functions (McCann 1982). Possibilities do exist that the insecticides may destroy hormonal homeostasis by suppressing GnRH release which may act directly on the gonadotropins to alter gonadotropin synthesis and secretion or indirectly by altering the pituitary cell responsiveness to GnRH through the actions of gonadal steroids resulting from alterations in FSH and LH by feed back mechanism (Stoker *et al* 1993).

OPs and CMs affect thermoregulation in birds. Acute sub lethal exposure to OP results in pronounced, short-lived hypothermia (Grue *et al* 1991). OP and CM induced reductions in body temperatures in birds are often associated with decreases in AChE activity of more than 50% (Clement 1991). The enhanced mortality in birds (i.e. *Falco brain sparverius*) is reported at sub lethal doses at thermo-neutral temperatures (Rattner and Franson 1984). The interaction between low temperatures and pesticide toxicity appears to be the result of the impairment of thermoregulation, causing inability of birds to withstand the cold (Martin and Solomon 1991).

OPs interfere with immune system response in animals through both anticholinergic and non-cholinergic pathways (Barnett and Rodgers 1994, Vial *et al* 1996). Sublethal exposure to chloropyriphos and methidathion to young chickens results in reduction in WBC, neutrophils and lymphocyte count (Obaineh and Matthew 2009).

Hematological responses of blue rock pigeon (*Columba livia* Gmelin) was studied by Mandal and Lahiri (1989) after oral administration of chlordane (a cyclodiene) fenitrothion (a phosphothioate) and carbaryl (a CM) for one week. Comparable hematological disorders were induced by these insecticides which include reduction in total count of peripheral erythrocytes, hemoglobin content, hematocrit and total cellularity of spleen. Total count of peripheral leucocytes, on the otherhand, increased with marked heterophilia together with lymphopenia and monocytopenia. Both bleeding and clotting time became conspicuously prolonged in the experimental birds. The results indicate potential to use hematological responses for rapid on the spot assay of insecticide toxicity in non-target animals.

Effect of pesticides was studied (Hussain *et al* 2006) by estimating the cholinesterase (ChE) enzyme activity in two insectivorous bird species *i.e*. jungle babbler (*Turdoides striatus*) and Indian wren warbler (*Prinia subjlava*) inhabiting the cotton based agro-ecosystem of Multan and the agro-ecosystem of Chakwal, an area where pesticide use is believed to be at very low scale. Morphometric data of the sampled populations revealed comparatively larger body weights in the birds captured from the control area as compared to those of the cotton area. There was suppression in the brain AChE enzyme activities in both the bird species captured during the cotton season as compared to the other sets of data *i.e.* non-cotton season and control values. The inhibition of ChE enzyme activity in jungle babbler was at 10.2% (cotton vs non-cotton), 29.6% (non-cotton vs control) and 36.8% (cotton vs control). In Indian wren warbler this inhibition was 6.2% (cotton vs non-cotton), 39.2% (non-cotton vs control) and 42.9%(cotton vs control). The present levels of ChE inhibition in jungle babbler and Indian wren warbler provided evidence that insectivorous birds inhabiting the cotton based agro-ecosystem of Punjab are at considerable level of threat from the pesticides in use. This preliminary report suggest further investigations on determination of pesticide residues level in the avifauna, reproductive potential and viability of the insectivorous bird species in this ecosystem. .

It is generally assumed that the toxicity of pyrethroid insecticides to birds is negligible, though few species have been tested. The oral acute toxicity of formulated beta-cyfluthrin was determined (Orduna *et al* 2011) for canaries (*Serinus sp.*), shiny cowbirds (*Molothrus bonariensis*), and eared doves (*Zenaida auriculata*). Single doses were administered to adults by gavage. Canaries were found to be substantially more sensitive to formulated beta-cyfluthrin (LD_{50} = 170 ± 41 mg/kg) than the other two species tested (LD_{50} = 2234 ± 544 mg/kg and LD_{50} = 2271 ± 433 mg/kg respectively).

While it might be expected that herbicides in general are less acutely toxic to birds (and other animals) than insecticides, some herbicides are lethal to birds in small doses. Dinoseb, a dinitrophenol herbicide that interferes with the basic energy metabolism in both plant and animal cells(US EPA 1986), kills wild birds at doses of 7 mg/kg and is as acutely toxic to birds as some of the most toxic insecticides. Paraquat, another herbicide that is highly toxic to humans and animals (US EPA 1987) kills adult birds at several hundred milligrams per kilogram, but can kill nestling kestrels at doses almost as low as dinoseb's lethal doses. Some nestlings died after being fed paraquat at 10 mg/kg (Hoffmann *et al* 1985). At least one documented bird kill has occurred following agricultural use of paraquat (Rivera 1973). The herbicide DNOC (dinitro-o-cresol), also used as an insecticide, fungicide, and defoliant (Meister 1991) is moderately toxic to birds. Pheasants, a jack- daw, a skylark, and wood pigeons were found dead in Great Britain following use of DNOC on wheat and corn fields (Rudd and Genelly 1956). Brodifacoum, a common rodenticide, is highly toxic to birds. It also poses a secondary poisoning hazard to birds that may feed on poisoned rodents (US EPA 1998). Scavenging birds such as red kites (*Milvus milvus*) are particularly at risk from secondary poisoning as a result of picking up dead rats, particularly those who have ingested the more toxic 'second-generation' rodenticides (Bjorck 2010). In 2005 in UK, 20 dead barn owls and ten kestrels reported to contain one or more anticoagulant rodenticides and six barn owls and five kestrels had residues in the potentially lethal range. It was concluded that rodenticides may have contributed to the death of one barn owl and two kestrels, based on the circumstances of death and examination of carcasses. Residues in five of 23 red kites found dead would be potentially lethal to barn owls, while 17 of these had residues of at least one rodenticide, and ten had residues from two or three rodenticides (Walker *et al* 2008). From dead tawny owls collected under the Predatory Bird Monitoring Scheme, 20% (and 33% of owl livers) contained residues of one or more rodenticides (Walker *et al* 2008).

Chapter III

MATERIALS AND METHODS

3.1 Study of feeding habits of birds

Feeding habits of common birds i.e. *Columba livia* (blue rock pigeon), *Streptopelia decaocto* (eurasian collared dove), *Psittacula krameri* (rose ringed parakeet), *Acridotheres tristis* (common myna), *Corvus splendens* (house crow), *Turdoides caudatus* (common babbler), *Bubulcus ibis* (cattle egret), *Vanellus indicus* (red wattled lapwing) and *Milvus migrans* (pariah kite) were observed randomly (8AM to 6 PM) from March 2010 to September 2011 in different agrifields, orchards, residences of PAU and Ludhiana city, dairy farm of GADVASU. For each bird species, five observations were taken per month. Feeding behaviour was noticed either with naked eye or with the help of binocular at a distance from the bird in order to avoid the disturbance to birds. Birds were observed for type of food material they consumed i.e. whether they were taking grains, seeds, insects, fruits etc. On the basis of these observations the birds were categorized as grainivorous, frugivorous, omnivorous, insectivorous or carnivorous.

3.2 Collection and identification of excreta of birds

For collection of excreta of different bird species, first of all their roosting , foraging and nesting sites were found in PAU (agrifields, orchards, residences), dairy farm of GADVASU, residences of Ludhiana city and Jagron Science college. Fresh and dry excreta samples of some birds (house crow, cattle egret) were collected by spreading the plastic sheets under trees of the roosting sites/ nesting sites and then collecting the excreta in sterile plastic bags with the help of spatula. Dry excreta samples (blue rock pigeon, eurasian collared dove) were collected from nests. These excreta samples were labeled appropriately with the source, time and date of collection and then stored in refrigerator for further use.

Physical analysis of excreta including texture, colour shape and weight per pellet of birds was also recorded.

3.3 Microbial analysis of Excreta Microbial analysis of Excreta:

3.3.1 Media and Reagents

The different media, broth and identification kit used in present studies were purchased from Himedia Laboratories Pvt. Limited, Mumbai and all the reagents of analytical grade were purchased from Qualigens Fine chemicals, Mumbai.

3.3.2 Moulds

3.3.2.1 Isolation and Characterization of Moulds

One gram of each sample was thoroughly mixed in 9 ml of 0.85% normal saline. Aliquots (0.2 ml) from serial logarithmic dilutions of each suspension were pipetted onto the surface of duplicate Petriplates containing sabouraud dextrose agar (SDA) supplemented with chloramphenicol (50µg/ ml) to suppress bacterial growth. The inoculum was spread evenly with a sterile T-shaped glass applicator. The Petriplates were incubated at 28°C for 5 days to 3 weeks. The mean colony count was determined and represented as colony forming units (cfu/ g) of each sample.

3.3.2.2 Maintenance of cultures

The stock cultures of moulds were maintained on SDA media and stored at 4ºC. Subculturing was done after every two weeks.

3.3.2.3 Identification criteria

The moulds isolated were identified by studying their colony and microscopic morphology as described by Mishra (2010) and published description of Cermeno *et al* (2006).

3.3.2.4 Colony morphology

To study colony morphology the isolates were grown on SDA at 28°C. The microscopic morphology was studied by examining cultures with Lactophenol Cotton Blue (LPCB) stain. For the preparation of slide the technique of McGinnis (1974) was used.

3.3.3 Yeasts

3.3.3.1 Isolation and Characterization of Yeasts

One gram of each sample and 10 g dry pigeon excreta was thoroughly mixed in 9 ml and 90 ml of 0.85% of normal saline respectively, mixed well, kept for 30 minutes . Aliquot (1.0) ml was spread on Petriplates containing Staib's media plates and kept at 28^0C. The presence of brown- pink coloured colonies (*Cryptococcus neoformans*) appearing on Staib's medium was noticed.

3.3.3.2 Identification criteria

The isolated colonies were further tested by negative staining with Indian ink to study the presence of capsule.

3.3.4 Bacteria

3.3.4.1 Isolation and Characterization of Bacteria

One gram of each sample was thoroughly mixed in 9 ml of MacConkey broth and kept at 37^0C for 24 hours for pre-enrichment of sample. Aliquots (0.1 ml) from serial logarithmic dilutions of each suspension were pipetted onto the surface of duplicate Petriplates containing MacConkey agar. The inoculum was spread evenly with a sterile T-shaped glass applicator. The Petriplates were incubated at 37°C for 24- 48 hours. The mean colony count was determined and represented as colony forming units (cfu/ g) of each sample.

3.3.4.2 Maintenance of cultures

The stock cultures of bacteria were maintained on MacConkey media and stored at 4ºC. Subculturing was done after every two weeks.

3.3.4.3 Identification criteria

The bacteria isolated were identified using Himedia *Enterobacteriaceae* identification kit.

3.4 Heavy metals analysis

3.4.1 Digestion of excreta samples

The dry excreta samples of different bird species (blue rock pigeon, eurasian collared dove, rose ringed parakeet, common myna, house crow, common babbler, cattle egret, red wattled lapwing and spotted owlet) were pooled , 0.3-0.5 g excreta sample taken and 4ml of conc. HNO_3 was added. A blank was also prepared without the excreta sample. These samples are then digested in Perkin's microwave at 121°C for 52 minutes. The final volume was made to 25 ml with distilled water and the solution was filtered.

3.4.2 Elemental analysis of digested excreta samples

The digested samples were analysed for heavy metals (As, Cd, Pb, Fe, Cr, Cu, Ni, Zn and Mn) by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICAP-AES) at Department of Soils , PAU, Ludhiana.

Among the various techniques of elemental analysis, Atomic Emission Spectroscopy, employing Inductively Coupled Plasma (ICP) as the source of energy has proved the most useful for the simultaneous determination of major, minor and trace elements in virtually all types of soil and plant materials with excellent sensitivity and emission stability.

An ICP is a high energy, optically thin excitation source. Power from a radio frequency genrator is coupled to a flow of ionized Argon gas inside a quartz tube encircled by an induction coil. To initiate the plasma, argon is ionized by a momentary high voltage discharge. The ionized gas passing through the high frequency magnetic field absorbs energy. This cause further local heating and ionization to form a ball of electrically conducting gas of plasma. Liquid samples in the form of an aerosol are injected into the high temperature environment of plasma. Here the analyte forms free atoms which emit characteristic spectra. More than 70 of the elements in the periodic table are capable of being determined by ICP.

Plasma is a major excitation mechanism which is produced electrically in a carrier inert gas such as nitrogen or argon. The energy level of argon makes this element especially well suited for plasma production and this gas has the added advantage of chemical inertness.

PLASMA can be defined as a neutral gas containing significant numbers of both positive and negative ions or free electrons. Plasma can only be created and maintained by the continued injection of enough energy to ensure the new ions are created fast enough to compensate for those that are continually recombining to form neutral atoms.

The ICP source consists of three concentric quartz tubes through which argon flows and a two- or three- turn gold plated copper coil surrounding the tubes near the upper end (Fig. 1).

Alternating current at a frequency of 27.14 MHZ and power levels upto several kilowatts is passed through the copper coil. Once some ions have been produced by means of an auxiliary spark circuit, a heavy current is caused to flow in a circular path in the ionized gas, powered by magnetic induction. This raises the temperature of the resulting plasma to some 10000K. This is far above the softening point of quartz. The plasma torch is protected from destroying itself by using the flow of argon itself as coolant. The bulk of the argon enters the outer tube at a tangential angle, so that it swirls through the annular space at high speed thus moderating the temperature. The hot plasma tend to stabilize in the form of a toroid at some little distance from the wall and this also serves to prevent overheating.

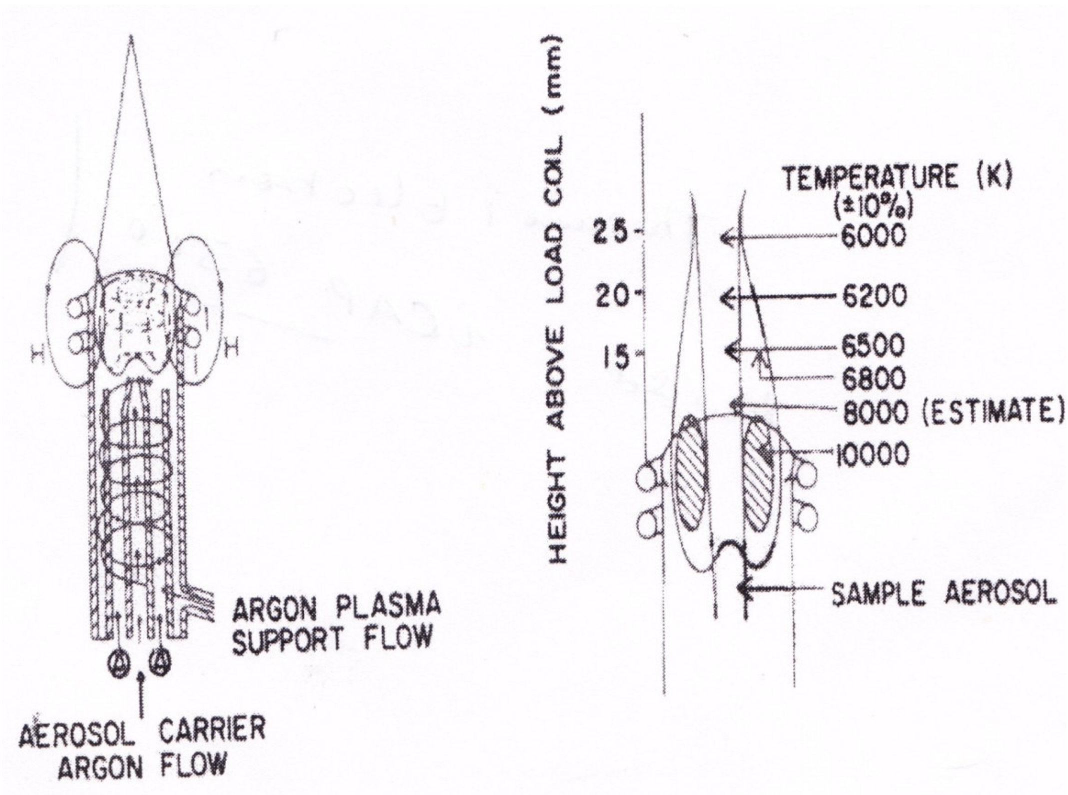


Fig 1: Schematic diagram of an ICAP-AES torch

The sample is aspirated in a neubulizer and is carried by a slower stream of argon directed centrally toward the "hole in the doughnut". Here the sample is heated by conduction and radiation and may reach 7000 K where it is completely atomized and excited. Loss of analyte atom by IONIZATION, a source of difficulty in flame and spark does not occur significantly in ICP spectroscopy, presumably because of the presence of the more easily ionized argon atom.

Spectrophotometers, using plasma as a source of energy are not inherently different from those using spark or arc. Some models operate in sequencial mode, wherein the wavelengths for all desired elements are scanned in order. Others act in a simultaneous mode in which many elements are detected at the same time with multiple photo tubes.

Calculations

The readings taken on ICAP-AES were converted into parts per million (ppm= µg/ g) as per the following formula:

$$\frac{\text{(Reading of sample – reading of blank) X Total volume used}}{\text{Weight of sample}}$$

3.5 Analysis of pesticide residue/ metabolites

3.5.1 Reagents and Chemicals

Acetone, dichloromethane, hexane, sodium sulphate and activated charcoal (all of LR grades) were redistilled in all glass apparatus. Running reagent blanks before actual analysis ensured the suitability of solvents and other chemicals.

3.5.2 Pesticide standards

The pesticide standards used in the study (mosty imported from M/s Dr. Ehrenstrorfer Gmbh, Ausburg, Germany while p,p'- DDE, p,p'- DDD, heptachlor, chlorpyriphos, quinalphos and triazophos standards obtained from Sigma Aldrich) were provided by the Department of Entomolgy, College of Agriculture, PAU, Ludhiana. The stock solutions of pesticides containing 1000µgm/l of analyte was prepared using acetone as solvent and kept at -20°C. Working solutions of 0.01, 0.05, 0.1, 0.5, 1.0 and 5 µgm/l were prepared by serial dilutions and stored at 4°C. Thses standard solutions were used for calibration purposes.

3.5.3 Extraction

5 gram samples of excreta of different bird species (blue rock pigeon, eurasian collared dove, common myna, house crow, common babbler, cattle egret and red wattled lapwing) were dipped in 50 ml acetone overnight. Filtered the extracts with rinsings of acetone in 500 ml separating funnel containing 250 ml of distilled water of 5% sodium chloride solution. 75 ml of dichoromethane was added and lower layer was collected. Then rinsed with 75ml of hexane twice and the layer above the aqueous layer was collected. The different fractions were combined and treated with 100 mg of activated charcoal powder for about 2-3 hours at room temperature. The clear extracts so obtained were filtered and concentrated in a rotary to 15 ml.

3.5.4 Clean Up

The extracts were cleaned up by column chromatography using silica gel (60-120 mesh) as an adsorbent. Before use the silica gel was activated at 110^{o}C for 2 hours. A glass coloumn (1.5 cm dia X 60 cm long) was packed with activated silica gel (20g+1g charcoal) in between the two small layers of anhydrous sodium sulfate supported on a plug of glass wool. The column was pre-washed with dichloromethane following which the extract was poured over it. The extract was eluted with a freshly prepared solvent mixture of dichloromethane-acetone (1:1 v/ v). The elute was concentrated to near dryness in a rotary evaporator under vacumm and then transferred to 5 ml acetone for further analysis.

3.5.5 Estimation

The sample extracts were analyzed using gas liquid chromatograph (GLC), model Nucon 5700 equipped with electron capture detector (ECD) and capillary column of 2m length X 3mm id packed with 1.5 % OV-17± 1.95% OV 210. The working conditions of GC included injector temperature 280^{o}C, column initial hold temperature 200 ^{o}C for 5 minutes, followed by 220 ^{o}C for 10 minutes and detected temperature 300 ^{o} carrier gas (nitrogen) flow was maintained 3.0 kg/cm^2 psi/g.

Before use, the column was primed with several injections of standard solution of either OCs or OPs pesticides till a consistent response was obtained. Suitable aliquots of cleaned sample were then injected into the ECD mode of the detector. The compound in the sample was indentified and quantified by comparisons of the retention time and peak heights of the sample chromatograms with that of standard run under identical operating conditions.

Calculations

The residues were quantified by using the formula below:

Residue level (ppm) =

$$\frac{\text{0.2 (NG used in standard)}}{\text{Area under peak with sample}} \times \frac{\text{area under peak with standard used}}{\text{volume of sample used}} \times \frac{\text{total volume made}}{\text{weight of sample taken}}$$

Chapter IV

RESULTS AND DISCUSSION

4.1 Food and feeding habits of birds

Feeding habits of nine bird species (blue rock pigeon,eurasian collared dove, rose ringed parakeet, common myna, house crow, common babbler, cattle egret, red wattled lapwing and pariah kite) were studied in different agrifields, orchards, residences of PAU and Ludhiana city, dairy farm of GADVASU.

4.1.1 Blue rock pigeon

The feeding habits of blue rock pigeon (*Columba livia*) was studied in agrifields of PAU; residences of PAU and Ludhiana city and it indicated that it feeds voraciously in flocks. The food consists of dried chapatis, dalia, soozi, wheat flour from residences; wheat, rice, bajra grains from residences as well as harvested agrifields and siliqua (fruit of *Brassica*) from agrifields (Table 1). The birds consumed large number of grains at a time when there was no disturbance around them.

4.1.2 Eurasian collared dove

The feeding behaviour of eurasian collared dove (*Streptopelia decaocto*) studied in agrifields of PAU showed that it feeds on wheat, rice, bajra grains and siliqua from harvested agrifields either solitary or in pairs alongwith flocks of blue rock pigeon. Both of these species are grainivorous (Table 1).

Bhattacharyya (1994) also reported that *Columba* and *Streptopelia* are ground feeders and predominantly grain-eaters, although quite often they invade diversified food-niches. The food of the blue rock pigeon chiefly consists of grains of rice, maize, millet and other cereals, pecked from the ground. This species also feeds on banyan figs and other berry-like fruits and sometimes insect eggs and larvae. Besides grains of paddy, wheat, millet, jowar (*Sorghum* sp.) and other cereals and pulses, the blue rock pigeon very often feeds on seeds of mustard, linseed and various weeds. Patel (2011) also observed that blue rock pigeon and Indian ring dove are grainivorous birds and they damaged sown seeds of sorghum and pearl millet while the rose-ringed parakeet was the major bird pest of millet crops (bajara and jowar) in Patan district of Gujarat. The pigeons feed on millet grains during the grain maturity stage, causing a considerable loss. However, Rana (1991) reported that the ring dove changes its food-habit quite frequently, depending on the availability of food as in the monsoon, the birds also feed on insects.

4.1.3 Rose ringed parakeet

Observations on feeding habits of rose ringed parakeet (*Psittacula krameri*) in agrifields and orchards of PAU indicated that this species feeds in flocks on seeds of *Brassica*, corn and bajra from harvested agrifields and mango, guava and other fruits in orchards and residences (Table 1, Plate I a). The rose ringed parakeets are frugivorous and gut contents recorded in adult birds contained maize grains, groundnut, guava fruits, mango, jamun, anthers and tree barks in different crop seasons (Kushwaha and Kumar 2007). Hussain *et al* (1991) revealed that flocks of parrots were observed to attack the guava fruits. The parakeets also feed the maize crop at both the milky and mature stages, removing the outer leafy foliage, consume the grains (Ramzan and Toor 1988). Although the parakeet possesses a wide feeding niche, yet it more explicitly attacks the oil seed crops viz. sunflower, brassica and canola, apart from a variety of orchards (Khan *et al* 2006). A loss of 25- 100% to *Brassica* mango, guava and sunflower by parakeet was also reported by Prasad and Verghese (1985) in India. Saini *et al* (1993) analyzed the gut contents of the rose ringed parakeets for one year and showed that it consisted of cereals (45%), tree orchards(38%) and oilseeds (16%). Ali (2002) reported that rose ringed parakeet often bands itself into large flocks and is highly destructive at all times to crops and orchard fruit by gnawing and wasting far more than it actually eats.

4.1.4 Common myna

The feeding behavior of common myna (*Acridotheres tristis*) studied in agrifields, orchard of PAU and residences of PAU and Ludhiana city indicated that it takes insects from grass ground, ploughed fields, water tunnels in wheat fields, irrigated rice fields; dried chapatis, rice grains, cooked rice, cockroaches from residences; corn and garbage waste (Table 1, Plate I b). The common myna either feeds in flocks or at least in pairs or is a surface feeder. This bird is omnivorous in nature. Common myna often feeds on different food items depending on the time of year perhaps due to the change in the availability of certain foods (Dhandhukia and Gadhvi 2010). Ali *et al* (2010) studied the regurgitated pellets of common myna which shows that insects were the main food of the common myna, constituting 92.40% (6,869 individuals) of the total number of prey items. Among insects, beetles (coleoptera) were dominant and comprised 24.17% (1003 individual remains) followed by grasshoppers (19.88%), bugs (16.33%) and bees, ants and wasps (13.49%). The other insects constituted <10% in the diet of the common myna. Ali *et al* (2010) also reported that common myna is a ground forager, which caught varieties of beetles, grasshoppers, ants, butterflies, etc. on the ground of the agricultural fields. Mathew *et al* (1978) reported that common myna was more abundant in rice fields and took many ground beetles and 53% of the stomach samples consisted of harmful insects of the order orthoptera, isoptera, coleoptera and hymenoptera. Common myna, bank myna, common babbler and rosy pastor were observed feeding mainly on insect pests of millet crops by Patel (2008) also. Mynas may consume grapes, apricots, apples, pears, strawberries, gooseberries and other fruit thus damaging the fruit crops (Brochier *et al* 2010). Mynas are omnivores (Sengupta 1976) and could potentially compete with a large range of indigenous species for food and also can cause severe damage to the

Table 1: Feeding Behavior of common birds

S. No.	Species	Areas observed	Type of food	Special feature of feeding Behaviour	Conclusion
1.	Blue rock pigeon (*Columba livia)*	Agrifields of PAU; Residences of PAU and Ludhiana city	Dried chapatis, dalia, soozi, wheat flour from residences; wheat, rice, bajra grains from residences as well as harvested agrifields, siliqua (fruit of Brassica) from agrifields	Feed in flocks, voracious feeder	Grainivorous
2.	Eurasian collared dove (*Streptopelia decaocto)*	Agrifields of PAU	Wheat, rice, bajra grains from harvested agrifields	Feeds as solitary or in pairs, feed alongwith flocks of blue rock pigeon	Grainivorous
3.	Rose ringed parakeet *(Psittacula krameri)*	Agrifields and orchard of PAU	Siliqua , corn, bajra; from harvested agrifieldsmango fruit, Guava,	Feed in flocks	Frugivorous
4.	Common myna (*Acridotheres tristis)*	Agrifields, orchard of PAU; Residences of PAU and Ludhiana city	Insects from grass ground, ploughed fields, water tunnels in wheat fields, irrigated rice fields; dried chapatis, rice grains, cooked rice, cockroach from residences; corn; garbage waste	Feed in flocks or at least in pairs, Surface feeder	Omnivorous
5.	House crow (*Corvus splendens)*	Agrifields, orchard of PAU; dairy farm of GADVASU, Residences of PAU and	Dried Chapatis and Bread; wheat grains in fields as well as residences, insects, larvae, earthworm from ploughed and irrigated fields;	Feeds as solitary as well as in flocks	Omnivorous

		Ludhiana city	ticks from buffalo ears; dead rats, dogs, owlets, pigeon etc; corn; mango fruit; garbage waste		
6.	Common babbler (*Turdoides caudatus)*	Agrifields, orchard of PAU; Residences of PAU and Ludhiana city	Dried chapatis and mulberry fruit from residences; insects lying on dry leaf debris in orchard and on boundaries of agrifields with low vegetation	Feed in flocks, Surface feeder	Omnivorous
7.	Cattle egret (*Bubulcus ibis)*	Agrifields of PAU and dairy farm of GADVASU	Ticks from buffalo ears; insects from irrigated wheat and rice fields as well as from ploughed fields	Feed individually as well as in flock of 4-5, usually feed with house crow and common myna following tractors in ploughed fields, neck movements while engulfing food	Insectivorous
8.	Red wattled lapwing (*Vanellus indicus)*	Agrifields of PAU	Insects from ploughed and irrigated fields	Feed as solitary or in flocks aligning themselves in a sraight line	Insectivorous
9.	Pariah kite (*Milvus migrans)*	Agrifields of PAU; Residences of PAU and Ludhiana city	Rat from residences and agrifields; garbage waste	Feeds as solitary as well as in flocks	Carnivorous

agricultural crops (Narang *et al* 1984) in the local community. Ali (2002) reported that common myna eats fruits, insects, kitchen scraps and follow the plough for earthworms. Sengupta (1976) reported that the principal diet of common myna consisted of grasshoppers, crickets, beetles, weevils, bugs, wasps and various other insects, their larvae and earthworm and this species consumed about 30 g of insects per day.

4.1.5 House crow

The feeding habits of house crow (*Corvus splendens*) were observed in agrifields, orchards, residences of PAU, dairy farm of GADVASU and residences of Ludhiana city which showed that it feeds on dried chapatis and bread in residences, wheat grains in fields as well as residences, insects, larvae, earthworm from ploughed and irrigated fields; ticks from buffalo ears; dead rats, dogs, owlets, pigeon etc; corn; mango fruit and garbage waste (Table 1, Plate I a, c and d). This species either feeds solitary or in flocks. This revealed the omnivorous nature of house crow. According to Khan *et al* (2007) the crow is a primary consumer and omnivorous in nature, foraging in small flocks throughout the day on farm crops, livestock farms, and in residential areas. The food of crow mainly comprises the kitchen refuse, fallen fruits in the gardens, and stored grains. The damage proportions of important foods by the crow indicates that the crow has acquired the status of a serious avian pest (Berruti 1990). According to Sen (2011) house crow destroys the food crops and the wheat grains in good numbers particularly at the post harvest level and therefore, directly competes with man for the food resources, but also causes significant economic losses (Dilshan *et al* 1982, Gourami 1985). Maize is one of the preferred food items for the house crow. House crows are omnivorous scavengers with a wide-ranging and opportunistic diet. They eat absolutely everything that is edible, and some that may not even be so. The crow feeds largely on refuse around human habitations, carrion, small reptiles, insects, small invertebrates, eggs, nestlings, plants, grain and fruits etc. Being closely associated with people (no populations are known to live independently of man), the crow takes advantage of scavenging opportunities provided by discarded food items and refuse dumps. This is particularly true in urban environments where other food sources like fruits, grain, insects, eggs etc. are limited. According to Ali (2002) house crow has no particular food preferences and eat almost anything, dead sewer rat, offal, carrion, kitchen scraps and refuse, locusts, termites, fruit, grain and eggs or fledgling birds pilfered from nests.

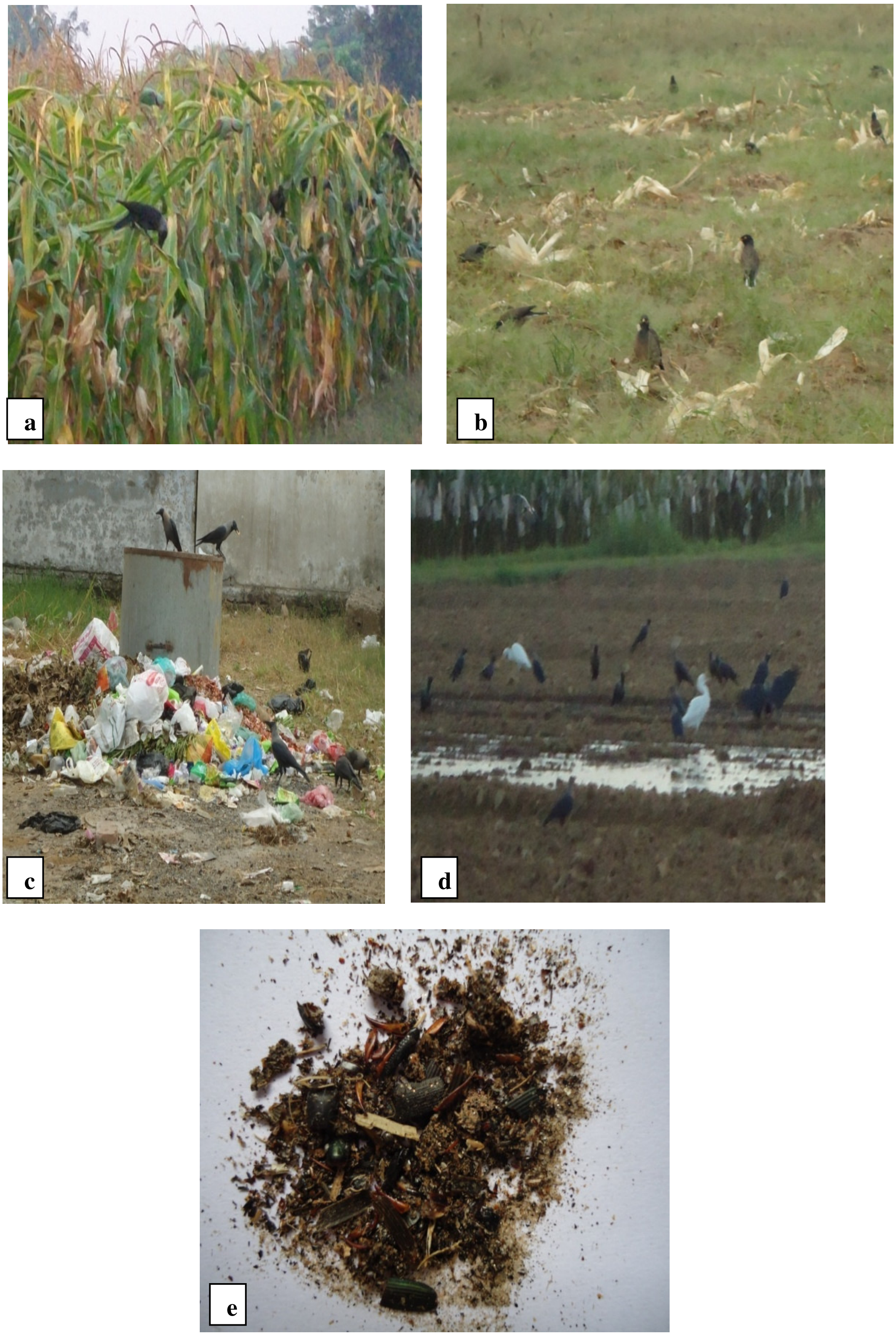
4.1.6 Common babbler

The observations on the feeding behaviour of common babbler (*Turdoides caudatus*) in agrifields and orchards of PAU as well as residences of PAU and Ludhiana city revealed that it feeds on dried chapatis and mulberry fruit from residences; insects lying on dry leaf debris in orchards and on boundaries of agrifields with low vegetation and it feeds in flocks

PLATE I: Observations on feeding habits of different Bird species

a. **Rose ringed parakeet and house crow feeding on corn**
b. **Common myna feeding in harvested corn fields**
c. **House crow feeding on garbage waste**
d. **House crow and cattle egret feeding in ploughed and irrigated fields**
e. **Regurgitated pellets of cattle egret showing presence of insect body parts**

PLATE I



(Table 1). This species is omnivorous. Ali (2002) reported that common babbler feeds on ground and scuttle along like rats through thorn, scrub and thickets and are loth to fly.

4.1.7 Cattle egret

The observations on the feeding habits of cattle egret (*Bubulcus ibis*) in agrifields of PAU and dairy farm of GADVASU showed that it takes ticks from buffalo ears; insects from irrigated wheat and rice fields as well as from ploughed fields (Table1, Plate I d) . This species feeds individually as well as in flocks of 4-5 and usually feeds with house crow and common myna following tractors in ploughed fields and the neck movements were observed while engulfing food. The regurgitated pellets collected from breeding sites of this species showed the presence of insect body parts (Plate I e). Thus the cattle egret is insectivorous in nature. According to Maddock (1990) the cattle egrets are insectivorous with almost 98% reliance on orthopterans in terms of prey occurrence in the guts. Nesting cattle egrets parents forage and capture any available insect to feed their off-springs (Jenni 1973). Ali (2002) reported that cattle egret is mostly seen with grazing cattle, stalking energetically alongside the animals, running in and out between their legs or riding upon their backs, and lunging out to seize insects disturbed by their movements amongst the grass. Mukherjee (2000) reported that cattle egrets which used to once feed in close association with grazing cattle have now shifted themselves behind tractors in inundated agricultural fields.

4.1.8 Red wattled lapwing

The feeding behavior of red wattled lapwing (*Vanellus indicus*) as observed in agrifields of PAU indicated that this bird species feeds as solitary or in flocks aligning themselves in a sraight line taking insects from ploughed and irrigated fields (Table 1). Thus, this bird species is also insectivorous in nature. Ali (2002) reported that the food of red wattled lapwing consists of insects, grubs, mollusks etc.

4.1.9 Pariah kite

The pariah kite (*Milvus migrans*) were also observed in agrifields of PAU as well as residences of PAU and Ludhiana city for their feeding behaviour. This species feeds as solitary as well as in flocks. The food comprised of rat from residences and agrifields; garbage waste (which contain some fleshy material). They were observed in large numbers in grounds and fields of PAU. Thus this species is carnivorous. Pariah kites have been recorded in large numbers on waste accumulations generated by human activities, including rubbish dumps, markets, fishing-ports and abattoirs all over their world range (Pomeroy 1975, Shiraishi *et al* 1990) and thus act as a scavenger. According to Ali (2002) the food of pariah kite comprised of offal and garbage, earthworms, winged termites, lizards, mice, disabled or young bird and almost anything else that can be procured. The rats and mice, which are forced to come out of their burrows when the harvested wheat and sugarcane fields are flooded with irrigation water, are caught by pariah kites and the house crows (Beg *et al* 2010).

4.2 Collection of Excreta of different bird species

The excreta collection of nine different bird species was done from the roosting , foraging and nesting sites of birds in PAU (agrifields, orchards, residences) and GADVASU (dairy farm) and outside PAU campus (residences of Ludhiana City and Jagron Science college).

4.2.1 Blue rock pigeon

The fresh excreta of blue rock pigeon was collected from roof windows in residences of PAU and Ludhiana city (Table 2). Here, these birds formed their nests. The fresh excreta of blue rock pigeon was also collected from the different agrifields (wheat, rice, bajra and *Brassica*) of PAU when the flock of this bird feed on the crop grains of these agrifields. The collection of excreta of blue rock pigeon was quite easy because their nests were easily located and moreover these birds feed in large flocks in the agrifields. The dry excreta of blue rock pigeon was collected from different agrifields as mentioned above as well as from the nests taken from roof windows of hostel No. 6 of PAU. The nests of blue rock pigeon contained a large quantity of faecal material (Plate II a). These nests were taken in October 2010. The collection of blue rock pigeon excreta was done from March to November(2010 and 2011).

4.2.2 Eurasian collared dove

Like the nests of blue rock pigeon, the nests of eurasian collared dove also contain a lot of fecal material. The defecation by doves on their nests may have adaptive significance, in that the excrement may strengthen their flimsy nests (Skutch 1964). The dry excreta of Eurasian collared dove was collected from nests taken from Ficus tree of PAU nursery in June, 2010 (Table 2).

4.2.3 Rose ringed parakeet

The collection of wet excreta of rose ringed parakeet was quite difficult as this bird species fly away very rapidly. The wet excreta of rose ringed parakeet was collected from *Brassica* agrifields of PAU when this bird species came to feed on Siliqua of *Brassica* crop This collection was done in March and April 2011. The dry excreta of rose ringed parakeet was collected from its cavity nest in Dhrek tree of PAU agrifields in October 2010 (Table 2).

4.2.4 Common myna

The wet and dry excreta of common myna was collected from water tunnels in the irrigated wheat agrifields of PAU in March and April 2011 (Table 2, Plate II b). This bird species came in large numbers on the water tunnels perhaps for taking the insects from flowing water.

4.2.5 House crow

The excreta of house crow was collected from different places as it is cosmopolitan. The wet and dry excreta was collected by spreading plastic sheets under mango trees in

Table 2: Sites and months of collection of excreta samples of different bird species

S. No.	Bird species	Excreta condition (Dry/ wet)	Source/ site of collection	Month of collection	Analysis done
1.	Blue rock pigeon	Wet	Residences and hostel of PAU and City residences.	March to November (2010 and 2011)	Microbial analysis
		Dry	Agrifields (wheat, rice, bajra, brassica) of PAU	March to November (2010 and 2011)	Microbial, heavy metals and pesticide residue analysis
			Nests taken from hostels of PAU	October 2010	Microbial, heavy metals and pesticide residue analysis
2.	Eurasian collared dove	Dry	Nests taken from Ficus tree of PAU nursery	June, 2010	Heavy metals and pesticide residue analysis
3.	Rose-ringed parakeet	Wet	Agrifields(Brassica) of PAU	March, April (2011)	Microbial analysis
		Dry	Nest cavities (Dhrek tree in agrifields of PAU)	October(2010)	Heavy metals and pesticide residue analysis of dry excreta
4.	Common myna	Wet and dry	Water tunnels in irrigated agrifields (wheat) of PAU	March, April (2011)	Microbial analysis of wet excreta and Heavy metals and pesticide residue analysis of dry excreta
5.	House crow	Wet and dry	1.Orchard (mango trees) 2. Agrifields (wheat, rice) 3. Dairy farm (trees) of GADVASU 4. Residences of PAU and outside PAU	March to November (2010 and 2011)	Microbial analysis of wet excreta and Heavy metals and pesticide residue analysis of dry excreta
6.	Common babbler	Dry	Trees (especially on branches) in orchards of PAU	March, April, June, September, October (2010)	Heavy metals and pesticide residue analysis
7.	Cattle egret	Wet and Dry	Tree (pilkan) having breeding colony of cattle egret at dairy farm of GADVASU	May and June (2010)	Microbial analysis of wet excreta and Heavy metals and pesticide residue analysis of dry excreta
8.	Red wattled lapwing	Wet and Dry	Water tunnels in irrigated agrifields (wheat) of PAU	March, April (2011)	Microbial analysis of wet excreta and Heavy metals and pesticide residue analysis of dry excreta
9.	Spotted Owlet	Dry	Dhrek tree at Jagron Science college	January and February (2010)	Heavy metal analysis

orchards of PAU (Plate II c) and pilkan tree of dairy farm of GADVASU (Plate II d), residences of PAU and city as well as from agrifields (wheat, rice) of PAU where these birds came for feeding (Table 2). Like blue rock pigeon, excreta of house crow was also collected from March to November (2010 and 2011).

4.2.6 Common babbler

The dry excreta of common babbler was collected from trees of orchard of PAU (Table 2, Plate II c). This bird species excretes on dividing branches of trees. Moreover, the excretory pellets of different birds of this species clubbed together and they were collected from under the trees of orchard. The collection was done in March, April, June, September and October (2010). The excreta eroded with rain water and thus could not be collected in July and August(2010).

4.2.7 Cattle egret

The wet and dry excreta of cattle egret was collected by spreading plastic sheets under Pilkan tree at dairy farm of GADVASU (Table 2, Plate II d). This tree was having breeding colony of cattle egret with more than 100 nests of cattle egret. The collection of excreta was done in May and June (2010).

4.2.8 Red wattled lapwing

The dry excreta of red wattled lapwing was collected from water tunnels in irrigated agrifields (wheat) of PAU in March and April (2011) (Table 2, Plate II b). As this bird species is insectivorous in nature so it came for taking the insects from irrigated agrifields.

4.2.9 Spotted owlet

The dry excreta of spooted owlet was collected from dhrek tree (where its nest cavity is present) in the month of January and February(2010) (Table 2).

4.3 Characteristics of excreta of different bird species

The excreta of different bird species was variable from each other in colour and shape. Initially, the excreta of each bird species was recognized by following the birds in the fields or collecting from their nests which made further collection easier.

4.3.1 Blue rock pigeon

The excreta of blue rock pigeon was semisolid in texture, green in colour when wet and turns white on upper side and dark green to black on lower side when dry. The shape was spiral and weight was 0.160± 0.02 grams/ pellet (Table 3, Plate III a).

4.3.2 Eurasian collared dove

The excreta of blue rock pigeon and eurasian collared dove resemble with each other as they belong to same order colombiformes thus providing information regarding their taxonomic relationships. The excreta of eurasian collared dove was also semisolid in texture, white on upper side and dark green to black on lower side when dry. The shape was also spiral and weight was 0.150± 0.01grams/ pellet (Table 3, Plate III b).

PLATE II: Different sources/ sites of excreta collection

a. **Nests of blue rock pigeon with excreta**
b. **Water tunnels in wheat agrifields**
c. **Trees of orchard at PAU**
d. **Pilkan tree of dairy farm at GADVASU**

PLATE II

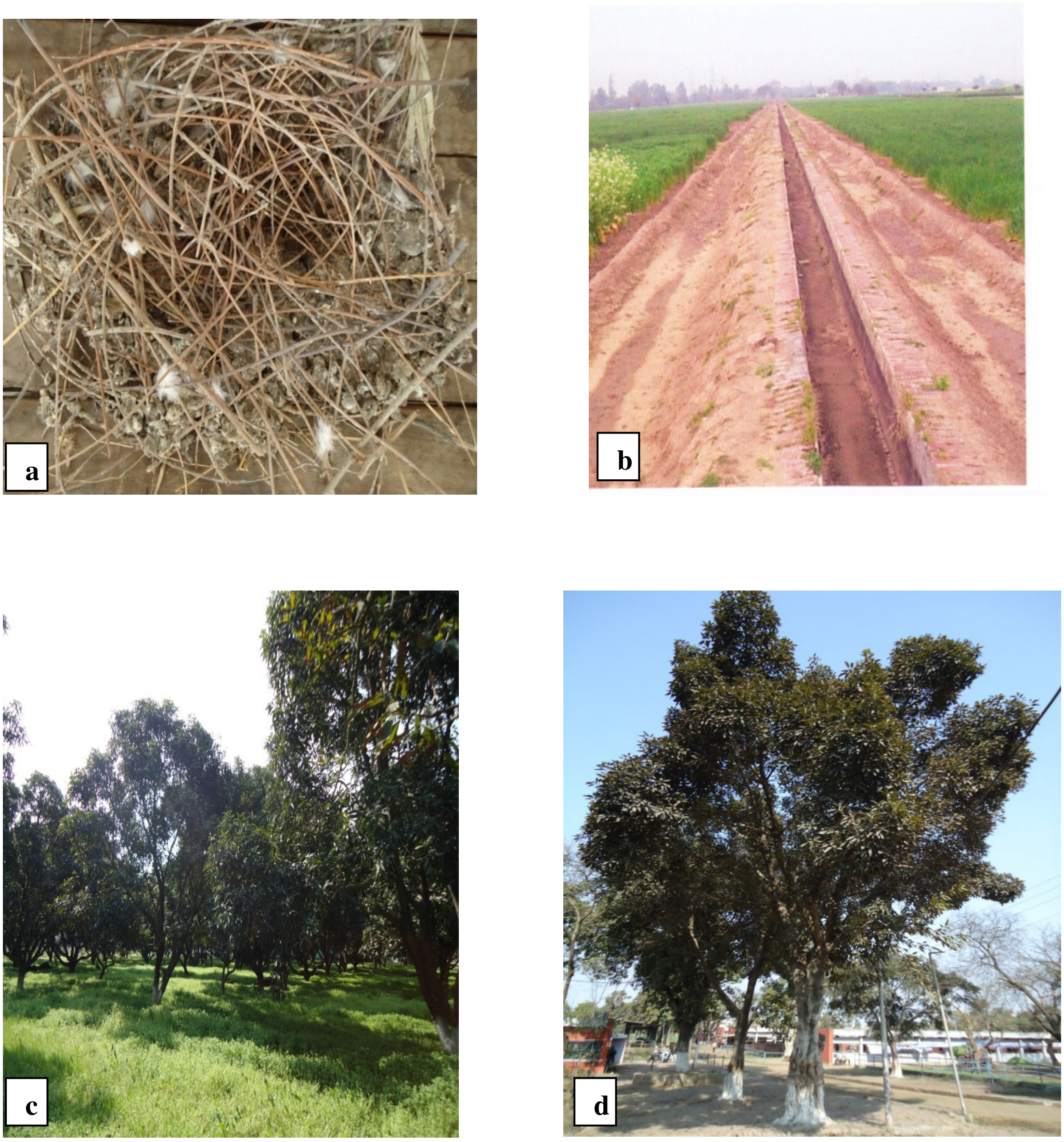


Table: 3 Characteristics of excreta of different bird species

S.No.	Bird species	Physical texture(wet)	Colour	Shape	[a]Weight (grams) of dry pellets
1.	*Blue Rock Pigeon	Semisolid	Green(wet) White on upper side and dark green to black on lower side(dry)	Spiral	0.160± 0.02
2.	*Eurasian collared dove	Semisolid	same as that of blue rock pigeon	Spiral	0.150± 0.01
3.	*Rose ringed parakeet	Semisolid	Green (wet as well as dry)	Irregular	0.204± 0.12
4.	*Common myna	Semisolid, sometimes with partly digested grains(also visible in dry excreta)	Varies from dark green to slightly brown(wet as well as dry)	Thin and rod shaped	0.158± 0.07
5.	*House crow	Semisolid, broken crop grains visible (also in dry excreta)	Varies from purple to brown(wet as well as dry)	Irregular	0.114± 0.01
6.	*Common babbler	Semisolid	White at one and and dark grey at other end(wet as well as dry)	Rod shaped	0.193± 0.03
7.	*Cattle egret	Semiliquid	Dark brown(wet as well as dry)	Irregular	0.248± 0.16
8.	*Red wattled lapwing	Semiliquid	Grey and white (wet) and Light grey (dry)	Rod shaped	0.294± 0.09
9.	Spotted owlet	Semisolid	Creamish white (dry)	Round	0.089± 0.08

weight of excreta of all bird species is expressed as mean± S.E. of 25* and 5** dry pellets
[a] Significantly differ in different species, $P \leq 0.05$.

4.3.3 Rose ringed parakeet

The excreta of rose ringed parakeet was semisolid in texture, green in colour in both wet and dry conditions, irregular in shape and weighed 0.204± 0.12 grams/ pellet (Table 3).

4.3.4 Common myna

The excreta of common myna was semisolid and sometimes with partly digested grains which were also visible in dry excreta. This depicted that the favorable food of common myna are insects. Sengupta (1976) also indicated that principal diet of common myna consisted of grasshoppers, crickets, beetles, weevils, bugs, wasps and various other insects, their larvae and earthworm and this species consumed about 30 g of insects per day. The crop grains as food passed out undigested in excreta of these birds. The color varies from dark green to slightly brown in both wet and dry conditions. The excreta was thin and rod-shaped. The weight was 0.158± 0.07 grams/ pellet (Table 3, Plate III c).

4.3.5 House crow

The excreta of house crow was semisolid and broken crop grains were visible both in dry and wet excreta. The color varied from purple to brown in both wet and dry conditions. The shape was irregular and weight was 0.114± 0.01 grams/ pellet (Table 3, Plate III d).

4.3.6 Common babbler

The excreta of common babbler was semisolid. The color being white at one and dark grey at other end in both wet and dry conditions. It was rod shaped. The weight was 0.193± 0.03 grams/ pellet (Table 3, Plate III e).

4.3.7 Cattle egret

The excreta of cattle egret was semiliquid and its color was dark brown in both wet and dry conditions. As the excreta was collected on plastic sheets falling from the nesting sites on Pilkan tree and thus it became irregular in shape on falling from trees. The weight was 0.248± 0.16 grams/pellet (Table 3, Plate III f).

4.3.8 Red wattled lapwing

The excreta of red wattled lapwing was semiliquid. The color was grey and white in wet condition and light grey in dry condition. It was rod shaped. The weight was 0.294± 0.09 grams/ pellet (Table 3, Plate III g).

4.3.9 Spotted Owlet

The excreta of spotted owlet was semisolid. The color was creamish white in dry condition. It was round shaped. The weight was 0.089± 0.08 grams/ pellet.

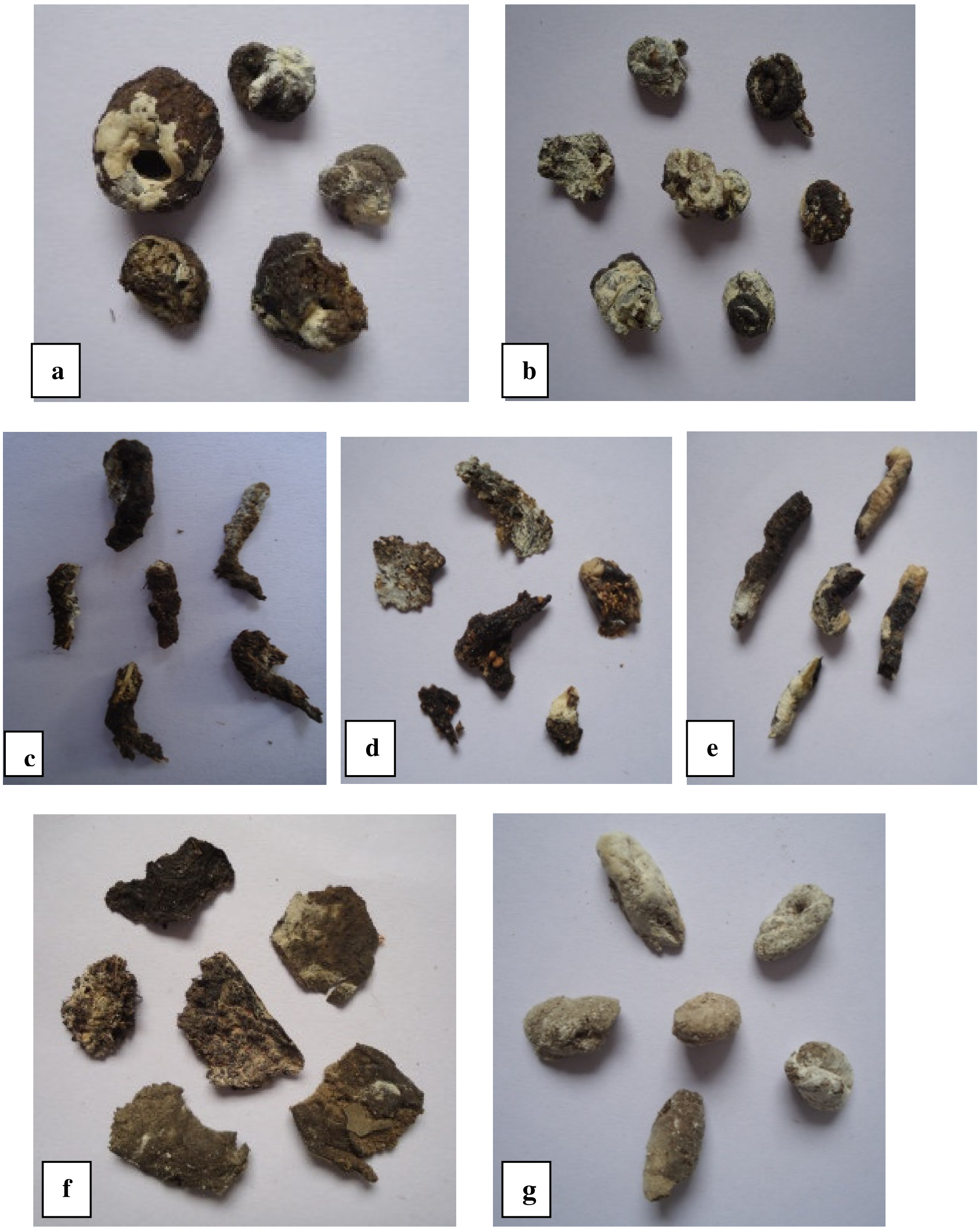
4.4 Microbial analysis of excreta of different bird species

The excreta of six bird species (blue rock pigeon, rose ringed parakeet, common myna, house crow, cattle egret and red wattled lapwing) was tested for the presence of fungi (moulds and yeast) and bacteria (*Enterobacteriaceae* family).

PLATE III : Excreta of different bird species

a. **Blue rock pigeon**
b. **Eurasian collared dove**
c. **Common myna**
d. **House crow**
e. **Common babbler**
f. **Cattle egret**
g. **Red wattled lapwing**

PLATE III



4.4.1 Moulds

The data on occurrence and population density of different kinds of moulds isolated from 15 excreta samples collected from about 80 pellets of six different bird species i.e. blue rock pigeon(n=5), rose ringed parakeet (n=1), common myna (n=1), house crow (n=5), cattle egret (n=2) and red wattled lapwing (n=1) are presented in Table 4 and the moulds isolated were *Aspergillus fumigatus, A.niger, A. flavus, Alternaria* sp. and *Geotrichum* sp. (Plate IV and Plate V). However, the excreta of different bird species varies in cfu/ g and percent isolation of these different moulds.

4.4.1.1 Blue rock pigeon

A total no. of five samples of excreta of blue rock pigeon were investigated for presence of moulds (Table 4). 3 (60%) yielded one or more moulds. *Aspergillus fumigatus, A. flavus* and *Alternaria* sp. were found to be commonest species occurring in 60% of the samples; followed by *A. niger* (40%) and *Geotrichum* sp. (20%) (Fig. 2). These results are in conformity with other reports (Kocan and Hasenclever 1972, Cermeno *et al* 2006). Emmons (1960) reported pigeon frequently has been associated with pathogenic and saprophytic fungi. Cermeno *et al* (2006) were able to isolate filamentous fungi such as *Aspergillus* spp (31.1%) in excreta samples of *Columbia livia* from several places in Bolívar state, Venezuela. The data on population density (cfu/ g) of moulds in the test excreta are presented in Table 5. Excreta of blue rock pigeon carried the highest population density of *Alternaria* sp. (11.7 X 10^5cfu/ g) followed by *A . fumigatus* (6.7 X 10^5 cfu/ g), *Geotrichum* sp. (5 X 10^5cfu/ g) , *A. niger* (2.5 X 10^5cfu/ g) and *A. flavus* (2 X 10^5cfu/ g).

4.4.1.2 Rose ringed parakeet

The population density of *A. fumigatus* was 5 X 10^5cfu/ g in excreta of rose ringed parakeet. *Aspergillus niger, A. flavus, Alternaria* sp. and *Geotrichum* sp. could not be detected (Table 5).

4.4.1.3 Common Myna

The population density of *A. flavus* and *Geotrichum* sp.was 5 X 10^5 cfu/ g and *A. fumigatus* was 2 X 10^5 cfu/ g of the excreta samples tested (Table 5). *Aspergillus niger* and *Alternaria* sp. could not be detected.

4.4.1.4 House crow

A total no. of five samples of excreta of house crow were investigated for presence of moulds (Table 4). *Aspergillus fumigatus* showed 100% recovery from excreta of house crow, followed by *A. niger* (60%) and *A. flavus* and *Alternaria* sp. (20% each) (Fig. 2). The

Table 4: Distribution of moulds in excreta of different bird species

S. No.	Bird species	Total no. of samples examined	Number of positive excreta samples				
			Aspergillus fumigatus	*Aspergillus niger*	*Aspergillus flavus*	*Alternaria* sp.	*Geotrichum* sp.
1.	Blue rock pigeon	5	3	2	3	3	1
2.	Rose ringed parakeet	1	*	*	*	*	*
3.	Common myna	1	*	*	*	*	*
4.	House crow	5	5	3	1	1	ND
5.	Cattle egret	2	2	1	1	1	1
6.	Red wattled lapwing	1	*	*	*	*	*

ND refers to not detected
*Only one sample tested, thus no intraspecies comparison made

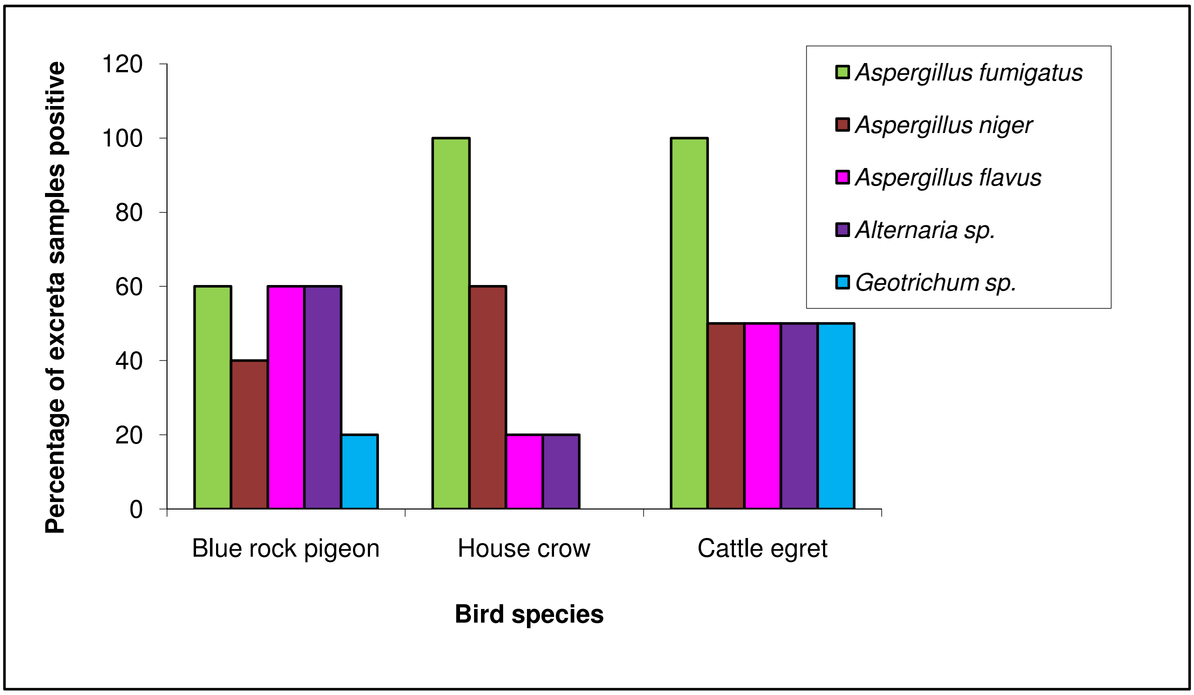


Fig. 2: Distribution of moulds in excreta of different bird species

excreta of house crow carried the highest population density of *A. fumigatus* and *Alternaria* sp. (5.2 X 10^5 cfu/ g followed by *A. niger* (3 X 10^5cfu/ g) and *A. flavus* (2.5 X 10^5cfu/ g) . *Geotrichum* sp. could not be detected (Table 5).

4.4.1.5 Cattle egret

Two samples of excreta of cattle egret was examined for presence of moulds (Table 4). *Aspergillus fumigatus* was present in both the samples indicating 100% recovery(Fig.2) whereas *A. niger, A. flavus* , *Alternaria* sp. and *Geotrichum* sp. could be isolated from only one sample indicating prevalence in 50% of the samples (Table 4, Fig. 2) .The population density of *Geotrichum* sp. was higher (2 X 10^4cfu/ g), followed by *A. fumigatus* (1 X 10^4 cfu/ g), *A. flavus* (2 X 10^3cfu/ g), *Alternaria* sp. (5 X 10^2cfu/ g) and *A. niger* (1 X 10^2 cfu/ g) (Table 5).

4.4.1.6 Red wattled lapwing

The population density of *Aspergillus fumigatus* was 8 X 10^6 cfu/ g followed by *A. flavus* 5 X 10^5cfu/ g and *A. niger* 6.5 X 10^4cfu/ g in excreta of red wattled lapwing. *Alternaria* sp. and *Geotrichum* sp. could not be detected (Table 5). Thus, the excreta of red wattled lapwing carries only *Aspergillus* species. The population density of *A. fumigatus* was highest in this species.

Table 5: Population density (mean cfu/ g of excreta) of moulds in different bird species

S. No.	Bird species	*Aspergillus fumigatus*	*Aspergillus niger*	*Aspergillus flavus*	*Alternaria* sp.	*Geotrichum* sp.
1.	Blue rock pigeon (n=5)	6.7 X 10^5	2.5 X 10^5	2 X 10^5	11.7 X 10^5	5X 10^5
2.	Rose ringed parakeet (n=1)	5 X 10^5	ND	ND	ND	ND
3.	Common myna (n=1)	2 X 10^5	ND	5 X 10^5	ND	5 X 10^5
4.	House crow (n=5)	5.2 X 10^5	3 X 10^5	2.5 X 10^5	5.2 X 10^5	ND
5.	Cattle egret (n=2)	1X 10^4	1X 10^2	2X 10^3	5 X 10^2	2 X 10^4
6.	Red wattled lapwing (n=1)	8 X 10^6	6.5 X 10^4	5 X 10^5	ND	ND

ND refers to not detected
cfu= colony forming units

Aspergillus fumigatus has been determined as the most pathogenic among the causative agents of aspergillosis with widespread resistant spores present in the natural environment (Raines *et al* 1956, Reece *et al* 1986, Akan *et al* 2002, Richard 1997). Aspergillosis is a respiratory tract infection caused by the genus *Aspergillus*, of which *A. fumigatus* is the causative agent of infections in wild birds. McDiarmid (1955) has shown that free-living wild birds become infected with *Aspergillus fumigatus* and other *Aspergillus* sp. causing aspergillosis which is a major cause of morbidity and mortality in birds (Tell 2005). Redig (1993) concluded that even simple colony is significant and should not be disregarded as an incidental finding. A wide variety of birds (waterfowl ,gulls, crows, raven, raptors, songbirds, upland gamebirds blackbirds, cowbirds, grackles, herons and shorebirds) have died of aspergillosis and probably all birds are susceptible to it. Young birds appear to be much more susceptible than adults (Friend 2001).

In the present studies, the excreta samples of all six species of birds contained pathogenic moulds. *Aspergillus* (especially *A. fumigatus*) was detected in all six species studied. House crow showed highest prevalence of *A. fumigatus* (100%) while the red wattled lapwing showed highest population density (8 X 10^6cfu/ g) of *A. fumigatus* thus depicting the harmful exposure of their spores in environment which may affect the other birds as well as including humans. This pathogen affects stressed and immunosuppressed animals (Briggs *et al* 1996, Carrasco *et al* 2001, Friend 2001). The prevalence of *A. fumigatus* was highest (86.7%) followed by *A. niger*, *A. flavus* (46.7%) , *Alternaria* sp. (33.3%) and *Geotrichum* sp. (20%) in the diverse excreta samples studied (Table 6, Fig. 3).

4.4.2 Yeasts

The 17 excreta samples of six different bird species i.e. blue rock pigeon(n=5, wet and n= 2, dry), rose ringed parakeet (n=1), common myna (n=1), house crow (n=5), cattle egret (n=2) and red wattled lapwing (n=1) were investigated for the presence of *Cryptococcus neoformans* on Staib's media. *Cryptococcus neoformans* was isolated in both wet and dry excreta of blue rock pigeon and in wet excreta of house crow (Table 7, Plate VI). *Cryptococcus neoformans* was not detected in wet excreta of rose ringed parakeet, common myna, cattle egret and red wattled lapwing . Earlier studies showed isolation of *C. neoformans* in old and dry excreta especially that of pigeon (Pal 1997, 2005) since dry excrement is a favorable substratum as it has fewer bacteria and therefore less competition, which could help explain the higher population density found in this substratum (Ruiz *et al* 1981). Old pigeon excreta probably remains the chief reservoir and source of *C. neoformans* (Pal 1997). Chee and Kim (2003) obtained *C. neoformans* isolates from withered pigeon droppings. Zarrin *et al* (2010) recovered *C. neoformans* environmental isolates from pigeon droppings in different urban areas in Ahwaz, Iran. Nawange *et al* (2011) showed that 37 samples(74%) out of 50 excreta samples collected from pigeon houses in Jabalpur (India) were positive for *C. neoformans*. Recently, Chowdary *et al* (2012) reported that *C. neoformans* showed a strong association with desiccated avian excreta. The reason for the

PLATE IV: Morphology of mould colonies isolated from excreta of different bird species

a. ***Aspergillus fumigatus***
b. ***Aspergillus niger***
c. ***Aspergillus flavus***
d. ***Alternaria*** **sp.**
e. ***Geotrichum*** **sp.**

PLATE IV

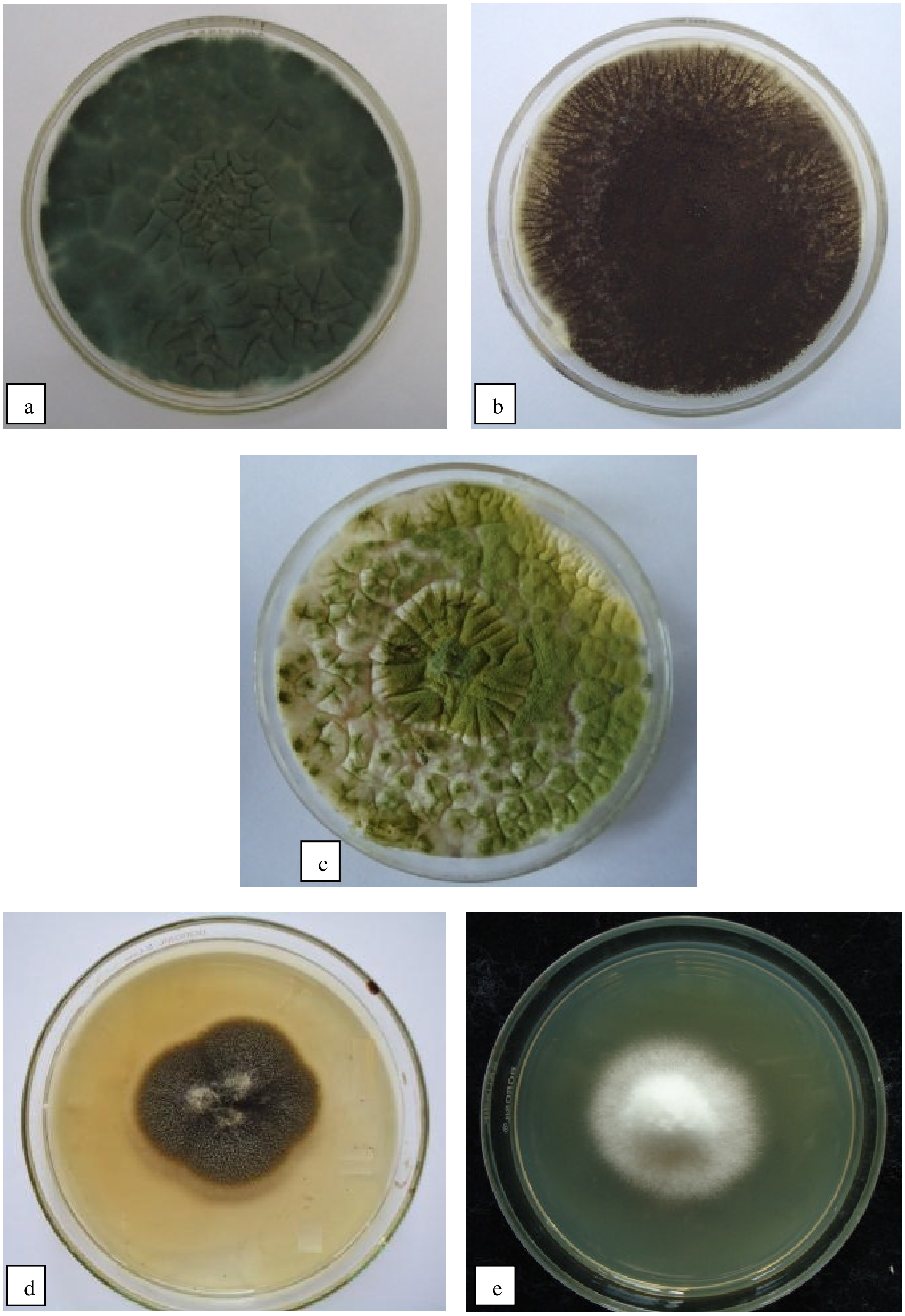


PLATE V: Slide culture preparation of mould colonies isolated from excreta of different bird species

a. ***Aspergillus fumigatus*** **(Magnification 400 X)**
b. ***Aspergillus niger*** **(Magnification 400 X)**
c. ***Aspergillus flavus*** **(Magnification 400 X)**
d. ***Alternaria*** **sp. (Magnification 400 X)**
e. ***Geotrichum*** **sp. (Magnification 400 X)**

PLATE V

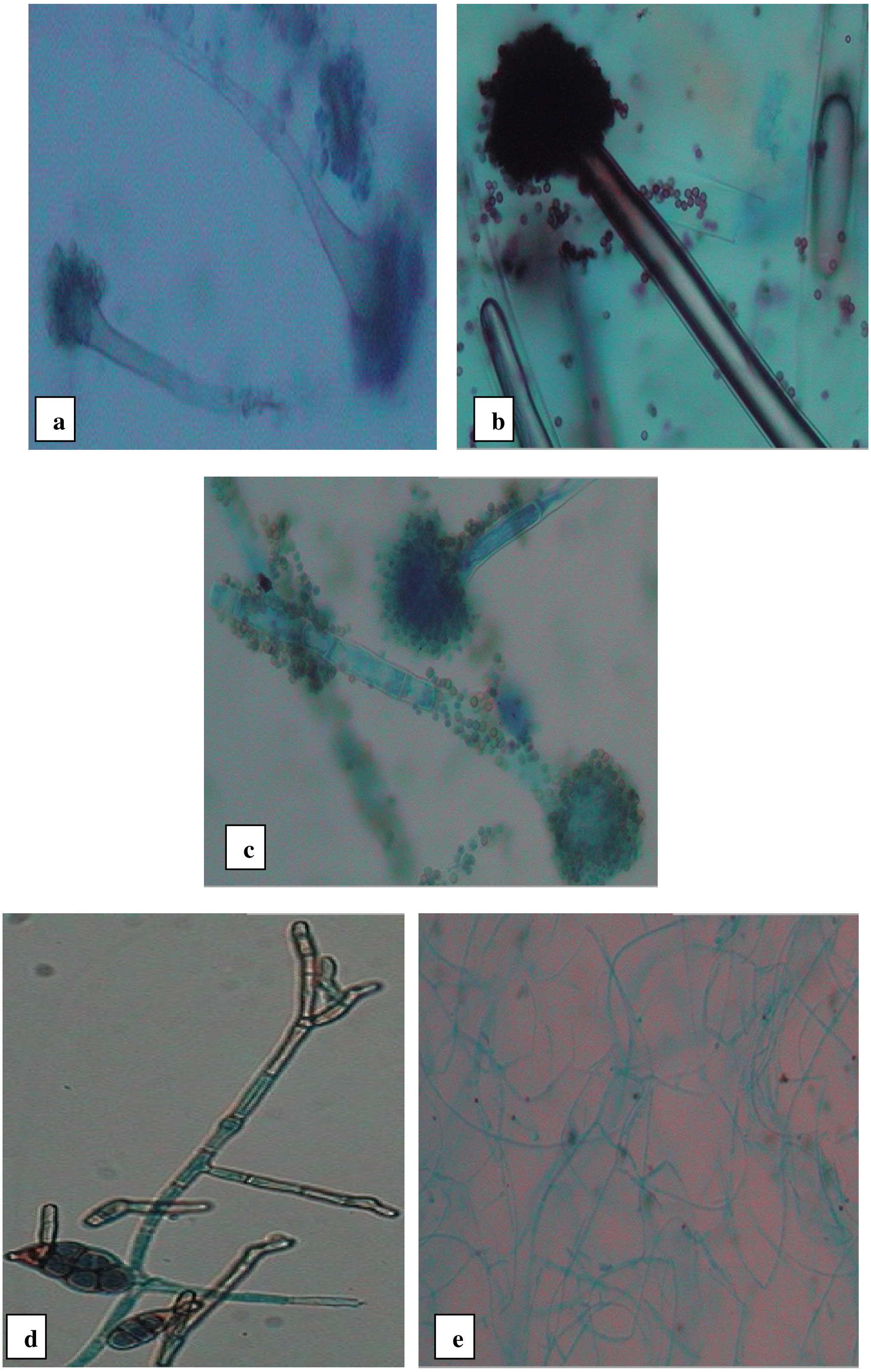


Table 6 : Percentage prevalence of moulds in excreta of different bird species.

S.No.	Mould colony	Percentage prevalence
1.	*Aspergillus fumigatus*	86.7
2.	*Aspergillus niger*	46.7
3.	*Aspergillus flavus*	46.7
4.	*Alternaria* sp.	33.3
5.	*Geotrichum* sp.	20

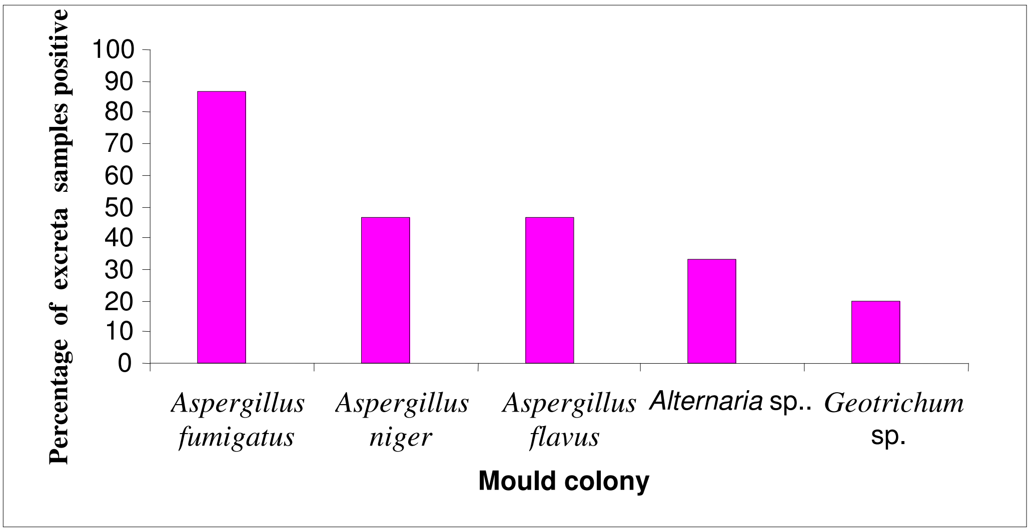


Fig. 3: Percentage prevalence of moulds in excreta of different bird species

Table 7: Occurrence of *Cryptococcus neoformans* in excreta of different bird species

S.No.	Bird species		*Cryptococcus neoformans*
1.	Blue rock pigeon	Wet (n=5)	+
		Dry (n=2)	+
2.	*Rose ringed parakeet (n=1)		-
3.	*Common myna (n=1)		-
4.	*House crow (n=5)		+
5.	*Cattle egret (n=2)		-
6.	*Red wattled lapwing (n=1)		-

* tested with wet excreta

+ refers to detected

- refers to undetected

high occurrence of *C. neoformans* in avian excreta is believed to be due to a utilization of nitrogenous compounds such as uric acid and creatinine from bird excreta (Lattman and Walter 1968). However, Mishra *et al* (1981) reported *C. neoformans*. was not found from fresh pigeon droppings and Pal (2005) recovered solitary isolate of *C. neoformans* from the fresh droppings of parakeets in Gujarat and eight isolates were obtained from the dry and old droppings.

4.4.2 Bacteria (*Enterobacteriaceae* family)

Few genus of bacteria were isolated from 12 excreta samples collected from about 60 pellets of six different species i.e. blue rock pigeon(n=4), rose ringed parakeet (n=1), common myna (n=1), house crow (n=3), cattle egret (n=1) and red wattled lapwing (n=1). This may be due to the reason bacteria need a temperature of 37 ° C for their growth, but in the present studies the collection is done in fields after the elimination from the bird body where the temperature is quite different from the growth temperature of bacteria. The amount of bacteria decreased with age of faeces. Moreover, the bacterial analysis of excreta is a sensitive test (Aho 1992)

The bacteria colonies were identified by biochemical characterization. These isolates could belong to *Citrobacter freundii, Enterobacter cloacae, Escherichia coli* and *Kleibsella pneumoniae* strains (Plate VII). As reported by Vilcins *et al* (2002) the members of the family *Enterobacteriaceae* including *Salmonella* species, *Escherichia coli*, *Enterobacter aerogenes* and *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus mirablis*, and *Providencia alcalifaciens* are pathogenic to humans. Thus excreta of all bird species studied in present work indicated a source of infection to humans.

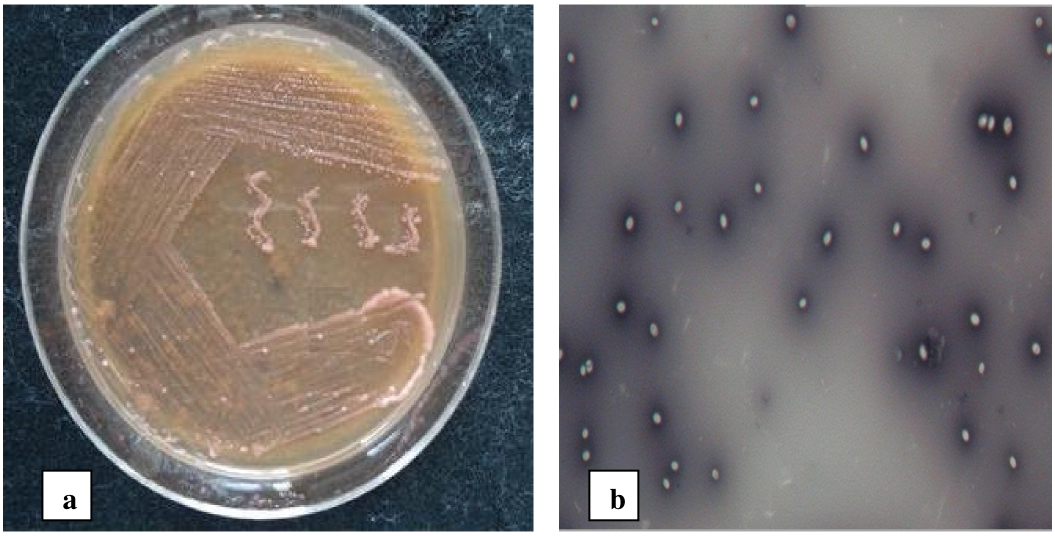
4.4.2.1 Blue rock pigeon

Out of the 4 samples of pigeon excreta samples tested for *Enterobacteriaceae* family, 3 were positive for *E. coli* indicating its prevalence in 75% of the samples and *C. freundii, E.cloacae* and *K. pneumoniae* was detected in only one sample revealing 25% of the samples showing positive isolation (Table 8, Fig. 4). The occurrence of *E. cloacae* and *E. coli* was highest (2 X 10^9 cfu/ g) followed by *K. pneumoniae* (1.2 X 10^9 cfu/ g) and *C. freundii* (8.2 X 10^8 cfu/ g) (Table 9). Abulreesh (2011) analyzed 400 fresh faecal samples of rock pigeons in Makkah city, western Saudi Arabia and reported 2.5% of samples to be positive for shiga-toxin producing *E.coli* 0157 . Shiga –toxin producing *E. coli* were found in faecal droppings and/or cloacal swabs of pigeons that live in urban and rural areas around the world (Haag-Wackernagel and Moch 2004, Wani *et al* 2004, Morabita *et al* 2001, Kobayashi *et al* 2007, 2009). Sonntag *et al* (2005) considered the pigeons as a possible reservoir of Shiga toxin 2f-producing *E. coli* associated with human disease. Ibrahim (2007) reported that bacterial pathogens including *Camylobacter jejuni, Citrobacter freundii, Diplococcus pneumoniae,*

PLATE VI: *Cryptococcus neoformans* isolated from excreta of birds

a. ***Cryptococcus neoformans* colonies**
b. **Capsule of *Cryptococcus neoformans* on India ink preparation (Magnification 400 X)**

PLATE VI



E.coli, Kleibsella oxytoca, K. pneumoniae, Mannheimia haemolytica, P. aeruginosa, Salmonella sp., *Staphylococcus aureus* and *Yersinia enterocolitica* were isolated from nasal and cloacal swabs of diseased pigeons.

Table 8: Distribution of bacteria in excreta of different bird species

S.No.	Bird species	Total no. of samples examined	Number of samples yielded			
			Citrobacter freundii	***Enterobacter cloacae***	***Escherichia coli***	***Kleibsella pneumoniae***
1.	Blue rock pigeon	4	1	1	3	1
2.	Rose ringed parakeet	1	*	*	*	*
3.	Common myna	1	*	*	*	*
4.	House crow	3	1	2	1	3
5.	Cattle egret	1	*	*	*	*
6.	Red wattled lapwing	1	*	*	*	*

ND refers to not detected,
*Only one sample tested, thus no intraspecies comparison made

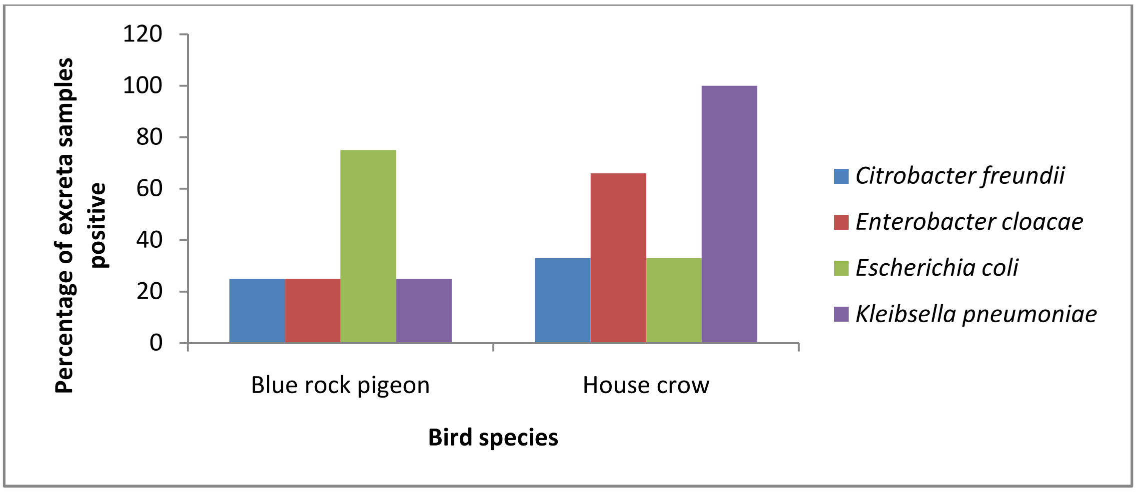


Fig. 4: Prevalence of bacteria in excreta of different bird species

4.4.2.2 Rose ringed parakeet

The excreta of rose ringed parakeet carried highest bacterial count of *E.coli* (7 X 10^9 cfu/ g) followed by *C. freundii* (6.5 X 10^9 cfu/ g), *K. pneumoniae* (2.7 X 10^9 cfu/ g) and *E. cloacae* (1.4 X 10^9 cfu/ g) (Table 9). Akhter *et al* (2010) also isolated *E.coli* (64%) from caged parrots of dhaka zoo of Bangladesh.

4.4.2.3 Common myna

The excreta of common myna carries highest bacterial count of *K. pneumoniae* (3.5 X 10^9 cfu/ g) followed by *E.coli.* (2.4 X 10^9 cfu/ g), *C. freundii* (1.4 X 10^9 cfu/ g) and *E. cloacae* (8 X 10^8 cfu/ g) (Table 9).

Table 9: **Population density (mean CFU/ g of excreta) of pathogenic bacteria in different bird species**

S.No.	Bird species	CFU/ g			
		Citrobacter freundii	***Enterobacter cloacae***	***Escherichia coli***	***Kleibsella pneumoniae***
1.	Blue rock pigeon	8.2 X 10^8	2 X 10^9	2 X 10^9	1.2 X 10^9
2.	Rose ringed parakeet	6.5 X 10^9	1.4 X 10^9	7 X 10^9	2.7 X 10^9
3.	Common myna	1.4 X 10^9	8 X 10^8	2.4 X 10^9	3.5 X 10^9
4.	House crow	3.4 X 10^8	1.7 X 10^9	3 X 10^8	2.3 X 10^8
5.	Cattle egret	1.7X 10^7	1.6X 10^9	2 X 10^8	ND
6.	Red wattled lapwing	1.2X 10^7	4 X 10^9	2.4 X 10^9	1X 10^9

ND refers to not detected
cfu= colony forming units

4.4.2.4 House crow

Out of the 3 samples of house crow excreta tested for *Enterobacteriaceae* family, 3 were positive for *K. pneumoniae* indicating its prevalence in 100% of the samples *E. cloacae* isolated from only 2 samples showing its prevalence in 66% of samples and *C. freundii* and *E.coli* was detected in only one sample revealing 33% of the samples showing positive isolation (Table 8, Fig. 4). The excreta of house crow carries highest bacterial count of *E. cloacae* (1.7 X 10^9 cfu/ g) followed by *C. freundii* (3.4 X 10^8cfu/ g), *E.coli* (3 X 10^8cfu/ g) and *K. pneumoniae* (2.3 X 10^8cfu/ g) (Table 9). Cooper (1996) isolated only *E. coli* and *Proteus* sp. from the rectum of Indian house crow. However, Yong *et al* (2008) identified 9 different species of bacteria belonging to the family *Enterobacteriaceae* (*Citrobacter freundii*, *Enterobacter cloacae*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Kluyvera ascorbata*, *Salmonella arizonae*, *Salmonella typhi*, *Shigella flexneri* and *Shigella sonnei*) in fresh faecal samples of the large-billed crow (*Corvus* sp.) collected from the study site at Bangsar, an urban setting in Kuala Lumpur, Malaysia. Crows being scavengers with very versatile feeding habits that will take food from the ground or might have fed on items which

PLATE VII: Biochemical characterization of Bacteria isolated from excreta of different bird species

- **a.** ***Citrobacter freundii***
- **b.** ***Enterobacter cloacae***
- **c.** ***Escherichia coli***
- **d.** ***Kleibsella pneumoniae***

PLATE VII


KB003 PART-I
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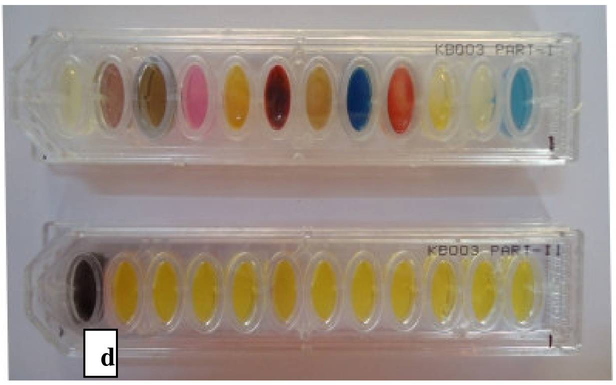
KB003 PART-II
a
30


KB003 PART-I
KB003 PART-II
b


KB003 PART-I
KB003 PART-II
c


KB003 PART-I
KB003 PART-II
d

have been contaminated by infectious droppings from another infected and become infected or be carriers of these pathogens.

4.4.2.1.5 Cattle egret

The excreta of cattle egret showed highest count of *E. cloacae* (1.6X 10^9 cfu/ g) followed by *E. coli* (2 X 10^8 cfu/ g) and *C. freundii* (1.7X 10^7 cfu/ g). *K. pneumoniae* could not be detected (Table 9).

4.4.2.1.6 Red wattled lapwing:

The excreta of red wattled lapwing carries highest population density of *E. cloacae* (4 X 10^9 cfu/ g) followed by *E. coli* (2.4 X 10^9 cfu/ g), *K. pneumoniae* (1X 10^9 cfu/ g) and *C. freundii* (1.2X 10^7 cfu/ g) (Table 9)

In the present studies, 73% of excreta samples showed presence of *E.coli* and 64% were positive for *C. freundii, E. cloacae* and *K. pneumoniae* (Table 10, Fig. 5). The population density of *C. freundii* was highest in rose ringed parakeet, *E. cloacae* in red wattled lapwing, *E.coli* in rose ringed parakeet and *K .pneumoniae* in common myna.

Table 10 : Percentage prevalence of bacteria in excreta of different bird species.

S. No.	Bacteria	Percentage prevalence of bacteria
1.	*Citrobacter freundii*	64
2.	*Enterobacter claocae*	64
3.	*Escherichia coli*	73
4.	*Kleibsella pneumoniae*	64

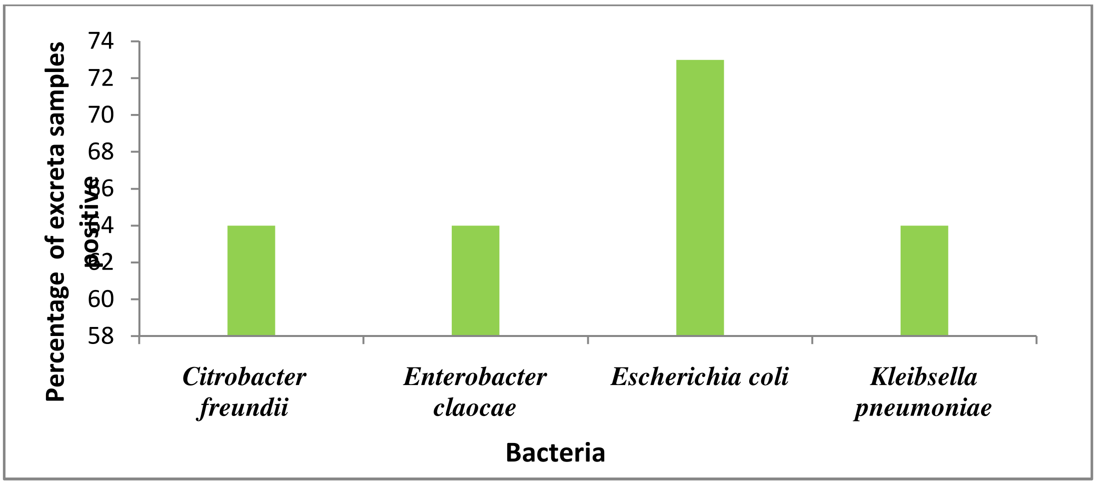


Fig. 5: Prevalence of bacteria in excreta of different bird spcecies

Thus from the microbial analysis of excreta of different bird species, it can be concluded that excreta of different bird species contains the wide range of pathogenic moulds, infectious yeasts and bacteria. As these birds are free living, so spreads of infectious agents occur through faecal contamination of drinking water sources, pastures and agricultural crops thus enabling the direct transfer of infectious agents to take place which may be a source of infection to human beings as well as may be harmful to birds themselves.

4.5 Heavy metal analysis in excreta of different bird species

The heavy metals studied in 5-15 pellets of different bird species (blue rock pigeon, eurasian collared dove, rose ringed parakeet, common myna, house crow, common babbler, cattle egret, red wattled lapwing and spotted owlet) in present studies included Arsenic (As), cadmium (Cd), lead (Pb), iron (Fe), chromium (Cr), copper (Cu), nickel (Ni), zinc (Zn) and and manganese (Mn). Out of these As, Cd and Pb are included in the category of non-essential elements and toxic whenever present. The others like Fe, Cr, Cu, Ni, Zn, Mn are essential elements needed in body to perform some specific functions. But all these essential elements are also toxic when present above threshold levels in human beings and birds too. The toxic heavy metals have been found to be present in excreta of all species (Table 11). The concentration of all heavy metals varied from species to species. The variation in concentrations of metal residues often attributed to interspecies differences (Gomez *et al* 2004, Taggart *et al* 2006). Discrepancies may be explained by dietary habits, habitat, excretion and/or absorption capacity of different species of birds (Elliott and Scheuhammer 1997). However, the presence of these heavy metals in excreta of different species of birds indicates that these birds are exposed to the heavy metals contamination in the environment. Faeces metal concenteration, however, tells less about tissue concenterations (Berglund 2010).

4.5.1 Arsenic (As) in excreta of different bird species

As is an element found in soil, water, air, plants and all living tissues. Large quantities of As is released into environment anthropogenically, primarily through agricultural practices including the application of pesticides. The concentration of As in excreta of different bird species was found to be 0.87±0.18 ppm in blue rock pigeon, 6.40±4.16 ppm in eurasian collared dove, 1.63±0.26 ppm in rose ringed parakeet, 8.92±3.35 ppm in common myna, 0.92± 0.25 ppm in house crow, 4.93± 3.61 ppm in common babbler, 2.36± 0.41 ppm in cattle egret, 7.51± 5.45 ppm in red wattled lapwing and 0.42 ppm in spotted owlet (Table 11, Fig. 6). The concentrations of heavy metals are usually low (1 ppm wet weight, which approximately represents 3 ppm dry weight) in most living organisms (Braune and Noble 2009). In the present studies the concentration of As was below this in excreta of blue rock pigeon, rose ringed parakeet, house crow and cattle egret and above this range in rest of the species i.e. eurasian collared dove, common myna, common babbler and red wattled lapwing. Thus, lowest concentration of As was in excreta of blue rock pigeon and it was its 2-fold in rose ringed parakeet, 3-fold in cattle egret, 6-fold in common babbler,

Table 11: Concentration of heavy metals (ppm) in excreta of different birds.

S. No.	Bird Species	Non essential elements			Essential elements					
		As	Cd[a]	Pb[a]	Fe	Cr	Cu	Ni	Zn	Mn
1.	[1]Blue rock pigeon	0.87± 0.18	0.28± 0.19	12.15± 3.22	1611± 519	3.42± 0.12	32.35± 17.73	9.98± 1.53	196.94± 113.53	128.66± 32.87
2.	[1]Eurasian collared dove	6.40± 4.16	2.73± 1.95	38.08± 23.42	19840± 13161	55.37± 41.08	85.49± 61.04	33.07± 19.42	412.36± 270.88	625.12± 399.09
3.	[2]Rose ringed parakeet	1.63± 0.26	2.13± 1.44	33.82± 23.56	8860± 575	29.94± 19.57	40.25± 20.20	24.71± 13.49	229.77± 153.06	323.77± 181.20
4.	[1]Common myna	8.92± 3.35	9.98± 1.41	122.14± 48.79	33788± 11856	109.16 ± 34.35	66.96± 19.52	53.78± 14.00	721.81± 125.84	1075.83± 151.92
5.	[1]House crow	0.92± 0.25	3.78± 2.85	61.71± 44.00	2455± 523	7.11± 3.96	44.13± 27.10	11.02± 7.49	358.61 ± 225.22	221.78± 112.33
6.	[1]Common babbler	4.93± 3.61	2.07± 1.38	25.86± 12.22	10793± 7753	33.77± 26.56	150.75± 110.58	19.20± 13.80	464.97± 314.10	401.43± 273.13
7.	[1]Cattle egret	2.36± 0.41	2.05± 0.91	7.23± 0.35	13223± 2588	23.45± 10.41	18.65± 2.49	10.83± 3.99	128.14± 8.06	242.31± 51.60
8.	[1]Red wattled lapwing	7.51± 5.45	48.79± 19.27	864.03± 383.83	29570± 13957	86.32± 31.46	87.43± 23.91	43.37± 14.01	473.99± 118.36	760.41± 402.43
9.	[3] Spotted owlet	0.42	4.17	90.49	10766	41.28	38.78	35.45	90.49	448.70

The data is mean ± S.E. of three[1] , two[2] and one [3]observations
[a] Significantly differ in different species (except spotted owlet as only one reading was available so not included for statistical analysis), $P \leq 0.05$.

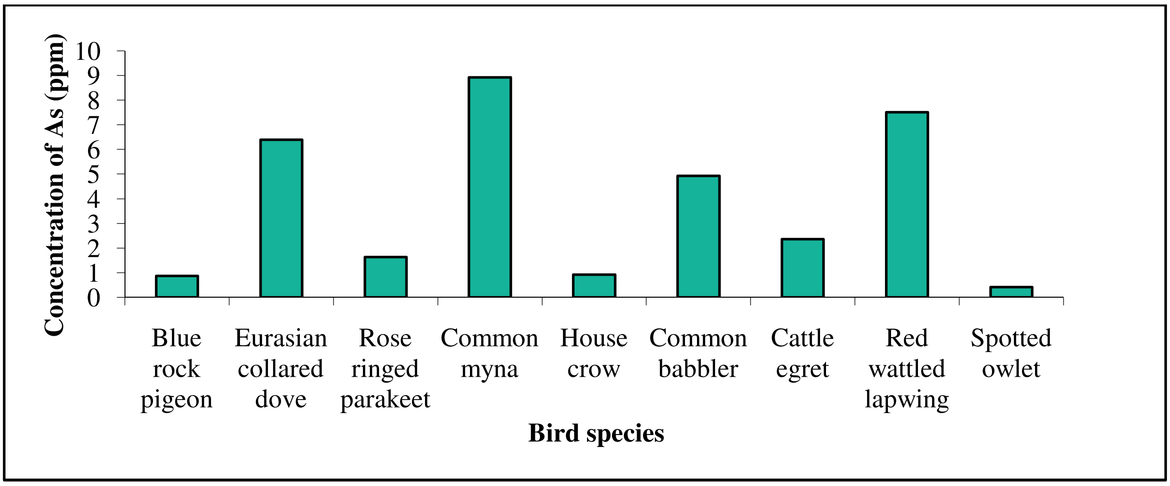


Fig. 6: Concentration of As in excreta of different bird species

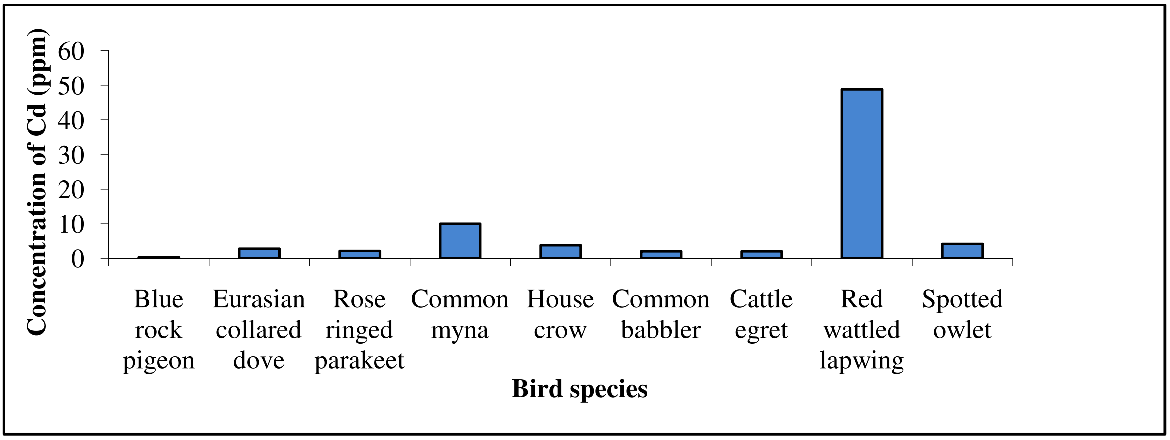


Fig. 7: Concentration of Cd in excreta of different bird species

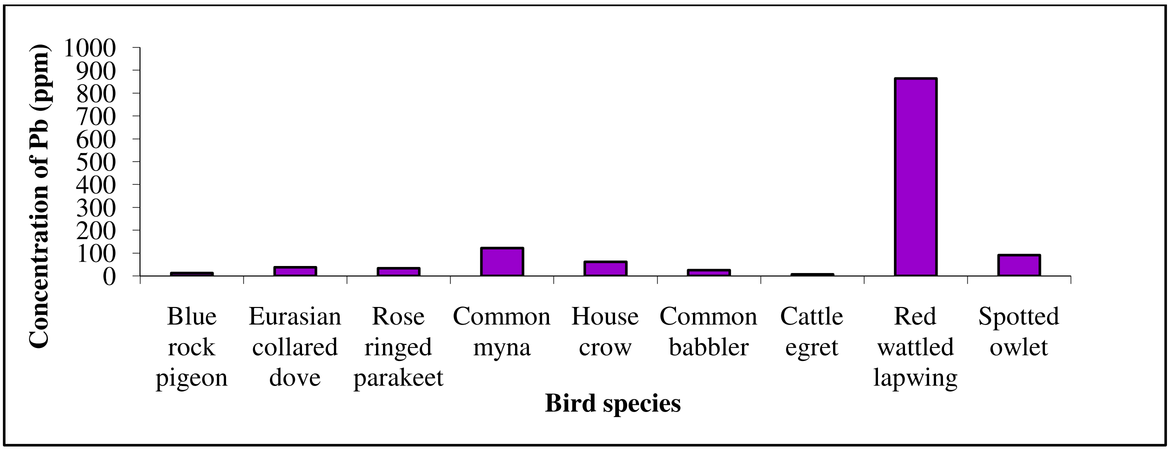


Fig. 8: Concentration of Pb in excreta of different bird species

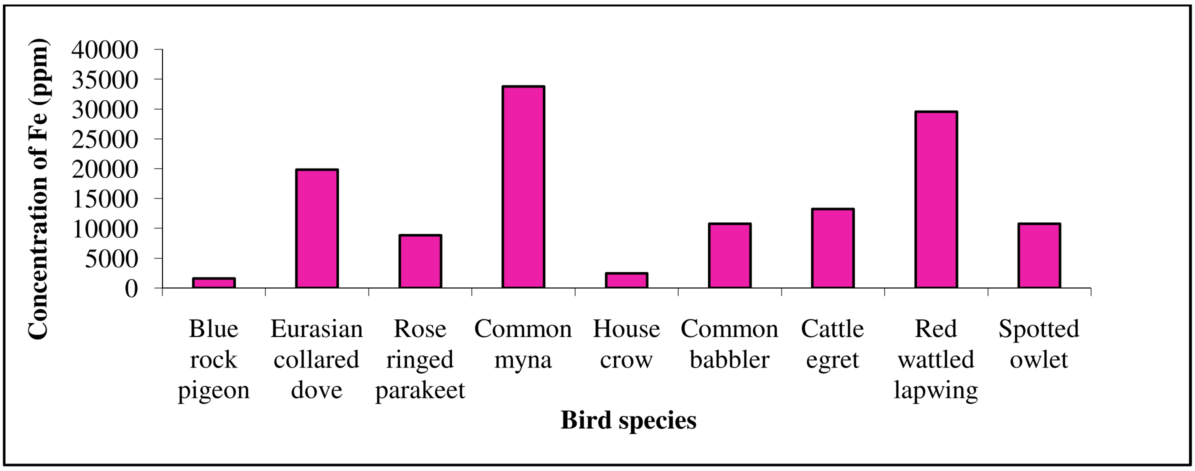


Fig. 9: Concentration of Fe in excreta of different bird species

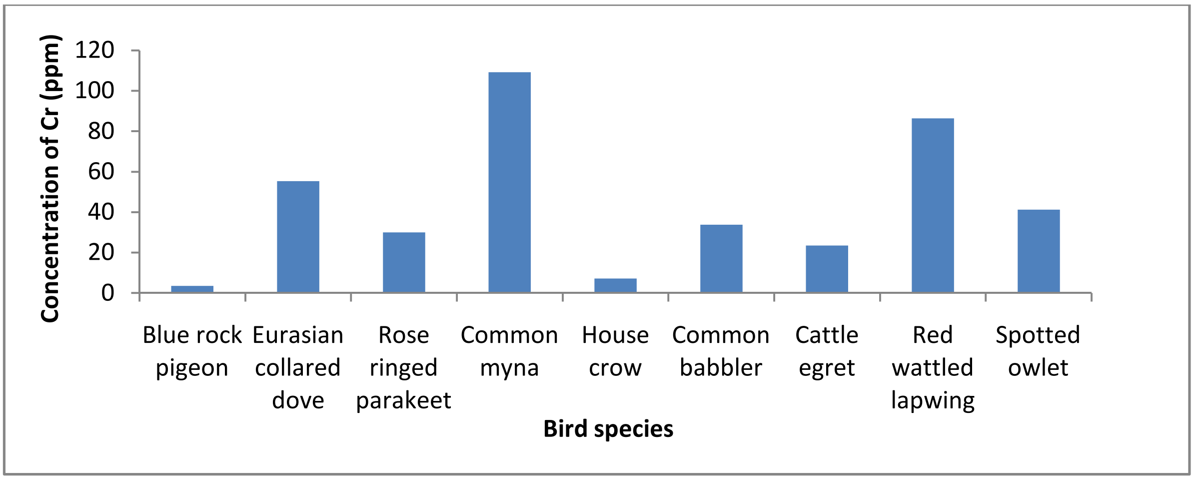


Fig. 10: Concentration of Cr in excreta of different bird species

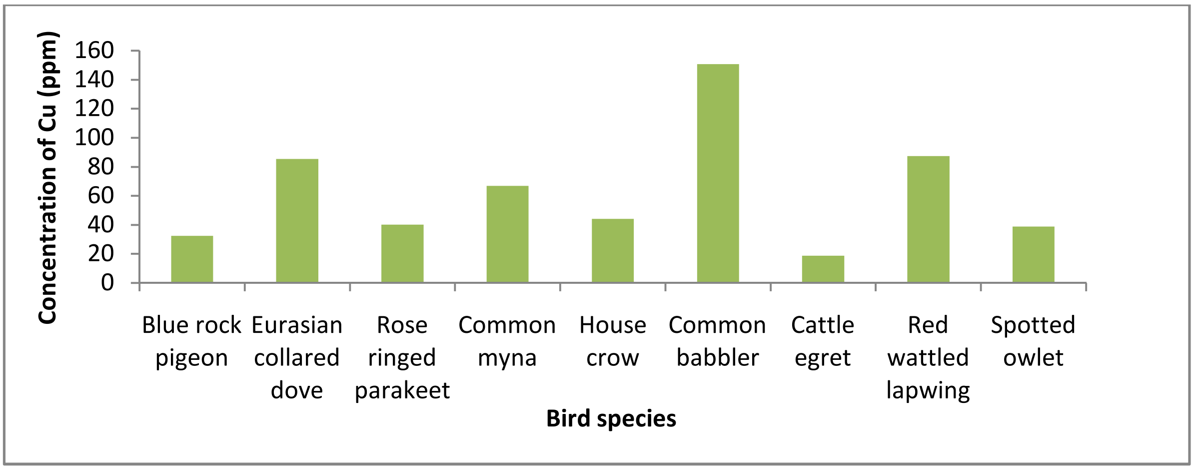


Fig. 11: Concentration of Cu in excreta of different bird species

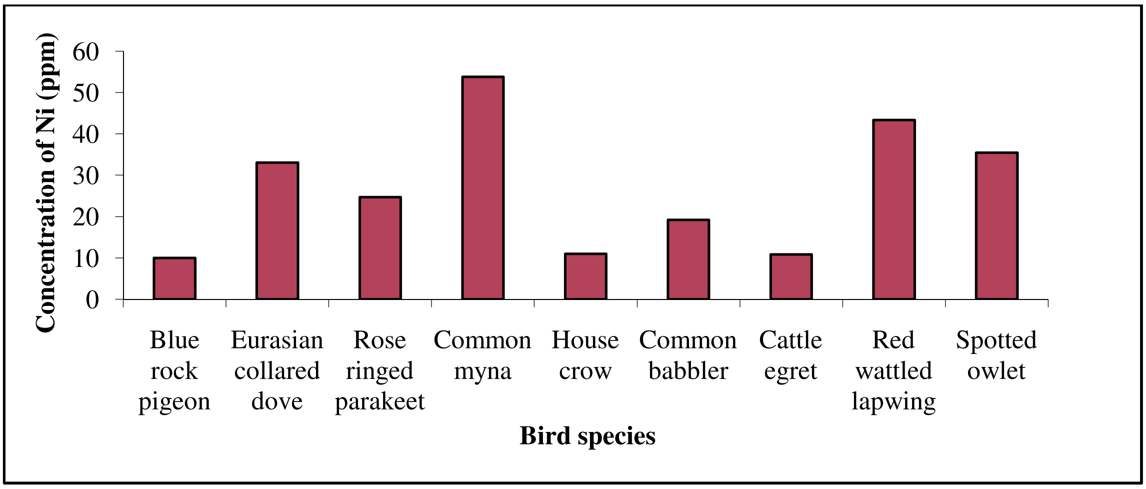


Fig. 12: Concentration of Ni in excreta of different bird species

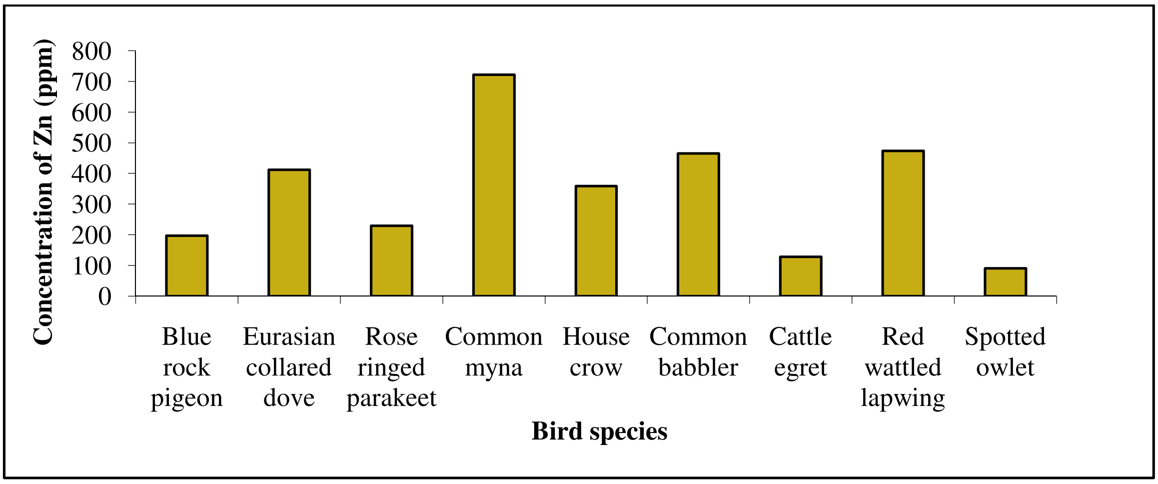


Fig. 13: Concentration of Zn in excreta of different bird species

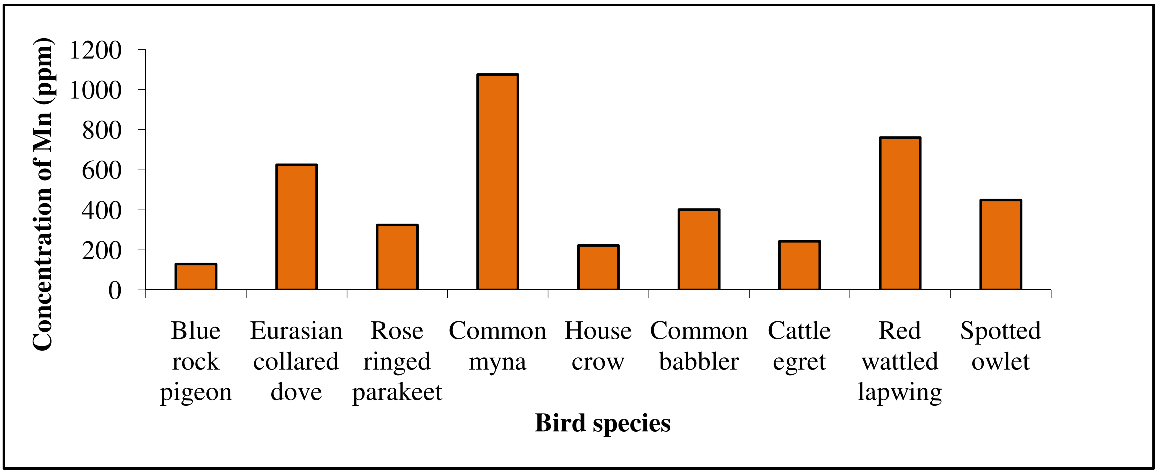


Fig. 14: Concentration of Mn in excreta of different bird species

7-fold in eurasian collared dove, 9-fold in red wattled lapwing and 10-fold i.e. maximum in common myna .Signs of acute arsenite poisoning in birds include ataxia, asthenia, slowness, jerkiness, falling, hyporeactivity, fluffed feathers, ptosis, huddled position, loss of righting reflex, immobility and tetanic seizures (Hudson *et al* 1984). Highly toxic inorganic As found in some seabirds may act as an endocrine disruptor, bring about the death of an individual, produce sublethal effects, or disrupt reproduction (Eisler 1994, Kunito *et al* 2008). Lebedeva (1997) found that the type of food eaten by birds plays a predominant role in the As amounts they would accumulate. However, observations in the present studies reveal that the grainivorous blue rock pigeon has lowest As in the faeces while the omnivorous common myna which lies high on a food chain has highest level of As but this gets greatly excreted in the faeces preventing it from its ill effects and thus helping this species to be dominant over others in agroecosystems as reported by Ishlash (2010). Despite Arsenic's reputation as a highly toxic substance, this element may actually be for the functioning of the nervous system and for proper growth.

4.5.2 Cadmium (Cd) in excreta of different bird species

Cd is one of the most abundant nonessential metals present in the environment because of its use in industrial activities. If absorbed into the organism through the digestive and pulmonary systems, cadmium forms complexes with proteins, in which it is easily transported and stored, mainly in the liver and kidneys and, in smaller quantities, in the pancreas, intestines and bones. Cd concentration was 0.28± 0.19 ppm in blue rock pigeon, 2.73± 1.95 ppm in eurasian collared dove, 2.13 ±1.44 ppm in rose ringed parakeet, 9.98± 1.41 ppm in common myna, 3.78± 2.85 ppm in house crow, 2.07± 1.38 ppm in common babbler, 2.05± 0.91 ppm in cattle egret, 48. 79± 19.27 ppm in red wattled lapwing and 4.17 ppm in spotted owlet (Table 11, Fig. 7) . The statistical data revealed that concentration of Cd varies significantly in 8 species studied. Cd concentration was lowest in excreta of blue rock pigeon and it was its 7- fold in common babbler and cattle egret, 8- fold in rose ringed parakeet, 10-fold in eurasian collared dove, 14-fold in house crow, 36-fold in common myna and 174-fold i.e. highest in red wattled lapwing. The high Cd accumulation in kidney demonstrates the role of this organ in the detoxification process and storage of nonessential elements. Lucia *et al* (2010) reported that kidney, followed by liver, was the main internal organ for Cd accumulation while muscle and feathers represented minor sites of Cd accumulation.This toxic metal is known to induce kidney toxicity, lesions in intestinal tissue, disruption of calcium metabolism, decreased food intake, and thinning of eggshells (Mayack *et al* 1981, Hughes *et al* 2000, Burger 2008). Battaglia *et al* (2005) and Toman *et al* (2005) inferred that kidney damage occurred in many species of birds when the Cd levels approached 20 ppm. Cadmium concentration above 100 ppm in kidneys has been suggested as a threshold concentration, above which Cd poisoning can be expected (Furness 1996). Burger (1993) suggested that feather levels of Cd that are associated with adverse effects would range from 0.1 ppb (shearwaters) to 0.2 ppb (terns). Bravo *et al* (2005) reported Cd level of 7.25 mg/l in plasma (2000 fold of Cd as found in human plasma) and high concentration i.e. 13.93 µg/ g in excreta of black vulture (*Coragyps atratus*). Thus the concentration of Cd in the present studies lies in toxic range in excreta of red wattled lapwing (48.79 ppm). However, Burger and Gochfeld (2000a) considered Cd concentration of 2µg /g as a threshold concentration in feathers that may have adverse effects in kidneys. The concentration of Cd was greater in excreta of 7 bird species (except blue rock pigeon) as compared to its concentration in feathers. Cd is also responsible for upsetting the activity of a number of cellular enzymes (Felsmann 1998, Alloway 1999). It produces tetratogenic, mutagenic and carcinogenic effects. Animals excrete more than 99% of the Cd they ingest mainly in the faeces (Lee *et al* 1994).

With continued exposure, even at low levels, this trace element is accumulated throughout the life span of birds (Dailey *et al* 2008). Kim and Koo (2007a) reported that cadmium contents in feathers of wild birds increased in relation to Cd concentrations in the diet. Greater concentrations of Cd in herons and egrets have also been associated to their feeding grounds (Kim and Koo 2007 b). Cd tends to bioaccumulate in food chain (Burger and Gochfeld 2004). Toxicological effects of Cd in birds have been reviewed by Furness (1996). Though, it is toxic above certain concentrations, Cd is not an essential element for animals and may induce deficiencies of essential elements through competition at active sites in biologically important molecules. At higher concentrations, it may cause kidney damage, altered behavior, suppression of egg production, egg shell thinning and testicular damage (Furness 1996). At the population level, reduced growth rates of bones and fledging success were correlated with exposure to elevated Cd concentrations in feathers (Spahn and Sherry 1999).

4.5.3 Lead (Pb) in excreta of different bird species

Sources of Pb in the environment include fuel additives, lead pigments in paints, batteries, pipes and glazed ceramic food containers. Inorganic Pb may be adsorbed through the skin, the respiratory system and gastrointestinal tract. Avian species are particularly at risk because they are exposed to Pb through ingestion of grit, soil intake from preening, or ingestion of contaminated food near ranges. Examination of the effects of Pb on birds at ranges have mainly focused on intake and toxicity of lead- shot pellets or fragments; however, Pb in soils may be an important pathway of exposure (Bannon *et al* 2011). The Pb concentration was 12.15 ± 3.22 ppm in blue rock pigeon, 38.08± 23.42 ppm in eurasian collared dove, 33.82± 23.56 ppm in rose ringed parakeet, 122.14 ± 48.79 ppm in common myna, 61.71± 44.00 ppm in house crow, 25.86± 12.22 ppm in common babbler, 7.23± 0.35 ppm in cattle egret, 864.03± 383.83 ppm in red wattled lapwing and 90.49 ppm in spotted owlet (Table 11, Fig. 8). The statistical analysis revealed that concentration of Pb significantly varies in 8 species. The concentration of Pb was lowest in excreta of cattle egret and it was its two fold in blue rock pigeon, 4-fold in common babbler, 5- fold in eurasian collared dove and rose ringed parakeet, 9- fold in house crow, 17- fold in common myna and 120- fold i.e. highest in red wattled lapwing . Scheifler *et al* (2006) indicated that Pb present in insects or prey items of birds may become available to birds in local environment. Thus this supported the observation of highest Pb level in excreta of insectivorous red wattled lapwing. Cattle egrets feed on insects on road where Pb deposition is likely to be high (Burger and Gochfeld 1997). For Pb, adverse effects in birds occur at levels of 4 ppm in feathers which were found to be associated with delayed parental and sibling recognition, impaired thermoregulation, locomotion, death perception, feeding behavior and lowered chick survival in gulls (Custer and Hoffman 1994, Burger and Gochfeld 2000b). Franson (1996) categorized liver tissue Pb levels of 2–6 µg/g to be subclinical exposure,3–6 µg/g to be toxic and 5 to 20 µg/g to be fatal in Galliformes, Falconiformes and Columbiformes respectively. Thus the concentration of Pb in the excreta of 8 species above these ranges indicating high exposure to the birds of 8 species to Pb . Tsuji *et al* (2002) recorded a range of Pb levels ≥20 µg/g indicative of Pb toxicosis in wild mallard ducks. Jayakumar and Muralidharan (2011) reported jungle babbler recorded the maximum concentrations (20.59 ± 9.07 µg/g) in muscle which falls in the toxic category. Pb which often is present in relation to hunting activities, impairs the growth and survival of nestlings; causes haemolytic anaemia in wild Pb-poisoned birds; has adverse effects on reproduction, such as decreased plasma calcium and egg production; and causes behavioural impairments (Scheuhammer 1987, Burger and Gochfeld 1994, Mateo *et al* 2003). Pb is known as calcium- formations seeking element, readily accumulates in bones, hairs, feathers and nails and is not metabolically regulated which makes it potentially important for monitoring of anthropogenic pollution (Metcheva *et al* 2006). Exposure to lead may cause kidney and nervous system problems. It can also inhibit heme synthesis (Berny *et al* 1994). Studies by Chaurasia *et al* (1997) showed that Pb deplete the essential thiol (- SH) groups causing disruption of type-I iodothyronine 5'-monodeiodinase (5'-D) enzyme configuration in the Indian rock pigeon, *Columba livia* leading to inhibition of deiodination of serum thyroxine (T4) to 3, 3', 5-triiodothyronine (T3). It has been suggested that birds may survive with high blood Pb levels without apparent symptoms of poisoning due to production of nuclear inclusion bodies (Tirelli *et al* 1996). It is also possible that Pb can be bound by metallothionein like proteins produced by red blood cells and sequestered in a non-bioavailable form, thus protecting an organism against Pb toxicity (Jayakumar and Muralidharan 2011). Pb may interact with calcium metabolism in birds (Hutton and Goodman 1980). Pb causes behavioral deficts in animals due to its toxic effects on the nervous system, and may result in decreases in survival, growth rates, poorer fledging success, learning and metabolism (Dauwe *et al* 2005, 2006). Higher incidence of mortality among the Pb-exposed nestlings was found (Spahn and Sherry 1999). Negative relationships between trophic position and metal levels have been found for Pb (Sydeman and Jarman 1998).

4.5.4 Iron (Fe) in excreta of different bird species

Fe is an essential part of hemoglobin in body. Fe is required for a number of vital functions, including growth, reproduction, wound healing, and immune function. The main role of Fe is to carry oxygen to the tissues where it is needed. Fe is also essential for the proper functioning of numerous enzymes involved in DNA synthesis, energy metabolism and protection against microbes and free radicals. Fe is released in the environment by steel industries. Fe concentration was 1611± 519 ppm in blue rock pigeon, 19840± 13161 ppm in eurasian collared dove, 8860± 5759 ppm in rose ringed parakeet, 33788± 11856 ppm in common myna, 2455± 523 ppm in house crow, 10793± 7753 ppm in common babbler, 13223± 2588 ppm in cattle egret, 29570± 13957 ppm in red wattled lapwing and 10766 ppm in spotted owlet (Table 11, Fig. 9) . Bravo *et al* (2005) reported the Fe concentration of 4823.76 ppm in excreta of black vulture (*Coragyps atratus*). Fe values excreted in human urine oscillate in the range of 60-100µg/ 24 hours. Fe concentration was also found to be lowest in excreta of blue rock pigeon and it was its 2-fold in house crow, 5- fold in rose ringed parakeet, 7-fold in common babbler, 8- fold in cattle egret, 12- fold in eurasian collared dove, 18- fold in red wattled lapwing and 21-fold i.e. highest in common myna.

4.5.5 Chromium (Cr) in excreta of different bird species

Cr is an essential mineral that plays a role with insulin to regulate blood sugar levels . Cr is found naturally in rocks, plants, soil and volcanic dust, humans and animals. Cr is generally produced by industrial processes. Cr concentration was 3.42± 0.12 ppm in blue rock pigeon, 55.37± 41.08 ppm in eurasian collared dove, 29.94± 19.57 ppm in rose ringed parakeet, 109.16± 34.35 ppm in common myna, 7.11± 3.96 ppm in house crow, 33.77± 26.56 ppm in common babbler, 23.45± 10.41 ppm in cattle egret, 86.32± 31.46 ppm in red wattled lapwing and 41.28 ppm in spotted owlet (Table 11, Fig. 10). Cr concentration was lowest in excreta of blue rock pigeon and it was its 2-fold in house crow, 7-fold in cattle egret, 9-fold in rose ringed parakeet, 10-fold in common babbler, 16- fold in eurasian collared dove, 25-fold in red wattled lapwing and 32-fold i.e. highest in common myna. According to Burger (1993) and Burger and Gochfeld (2000a) Cr concentration of 2.8µg/ g in birds feathers might be associated with adverse effects. Studies by Malik and Zeb (2009) showed high Cr concentration (6.6µg/ g to 7.12/ g) in feathers of cattle egret . Thus this provide information that Cr lies in high concentrations in excreta of 8 bird species. According to Kertesz and Fancsi (2003) Cr produces adverse effects on embryonic development, hatching and viability of the mallard.

4.5.6 Copper (Cu) in excreta of different bird species

Cu is involved in the formation of several key enzymes for the release of energy inside the cell, and contributes to the function of many antioxidants. Cu is widely used in industries and agriculture. Due to this, Cu quantities in the environment have increased. The copper concentration was 32.35± 17.73 ppm in blue rock pigeon, 85.49± 61.04 ppm in eurasian collared dove, 40.25± 20.20 ppm in rose ringed parakeet, 66.96± 19.52 ppm in common myna, 44.13± 27.10 ppm in house crow, 150.75± 110.58 ppm in common babbler, 18.65± 2.49 ppm in cattle egret, 87.43± 23.91 ppm in red wattled lapwing and 38.78 ppm in spotted owlet (Table 11, Fig. 11). Cu concentration was lowest in cattle egret excreta and it was its 2-fold in blue rock pigeon, rose ringed parakeet and house crow, 4 fold in common myna, 5-fold in eurasian collared dove and red wattled lapwing and 9 fold i.e. highest in common babbler. Bravo *et al* (2005) reported Cu level of 20.26 µg/ g in excreta of black vulture (*Coragyps atratus*) which lies in non-toxic range. Acute Cu poisoning occured in liver of canada goose (*Branta canadensis*) at Cu concentrations of 187 to 323 µg/ g (Henderson and Winterfield 1975). More than 100 µg/g of Cu in liver tissues of mute swan did not exhibit any sign of Cu toxicity (Jayakumar and Muralidharan 2011). Thus the toxic concentration of Cu existed only in excreta of common babbler (150.75 ppm). At high doses, essential elements, such as Cu and Zn could also have toxic effects on kidneys and impair reproduction (Heinz *et al* 1989, Carpenter *et al* 2004). Haemoglobinaemia, haemoglobinuria, jaundice and liver problems, are symptomatic of Cu poisoning (Larbier and Leclercq 1995, Danczak *et al* 1997).

4.5.7 Nickel (Ni) in excreta of different bird species

Ni plays a role in proper functioning of liver in the animals. Ni is one of many trace metals widely distributed in the environment, being released from both natural sources and anthropogenic activity, with input from both stationary and mobile sources.Environmental sources of lower levels of Ni include tobacco, dental or orthopaedic implants, stainless steel kitchen utensils and inexpensive jewellery. Ni concentration was 9.98 ± 1.53 ppm in blue rock pigeon, 33.07± 19.42 ppm in eurasian collared dove, 24.71± 13.49 ppm in rose ringed parakeet, 53.78± 14.00 ppm in common myna, 11.02± 7.49 ppm in house crow, 19.20± 13.80 ppm in common babbler, 10.83± 3.99 ppm in cattle egret, 43.37± 14.01 ppm in red wattled lapwing and 35.45 ppm in spotted owlet (Table 11, Fig. 12). Mean Ni concentration in feathers of cattle egret from Pakistan was recorded to be varied from 7.8µg/ g to 9.0µg/ g revealing the toxic Ni concentration (Malik and Zel 2009). Thus in the present work Ni concentration was found to be higher than as reported by Malik and Zeb (2009) in feathers of cattle egret revealing the high exposure of Ni in 8 bird species. Bravo *et al* (2005) reported Ni concentration of 15.19 µg/ g in excreta of black vulture (*Coragyps atratus).* Ni concentration was lowest in excreta of blue rock pigeon and it was its 2-fold in common babbler, 3-fold in eurasian collared dove and rose ringed parakeet, 4-fold in red wattled lapwing and 5-fold i.e. highest in common myna. In Ni-contaminated areas, Ni concentrations were elevated in feathers, eggs, and internal tissues of birds when compared to conspecifics collected at reference sites (Darolova *et al* 1989, Outridge and Scheuhammer 1993).

4.5.8 Zinc (Zn) in excreta of different bird species

A component of the enzymes responsible for metabolism of proteins and carbohydrates, Zn is an essential element in the animal organism. It is released to the environment from both natural and anthropogenic sources; however, releases from anthropogenic sources are greater than those from natural sources. The primary anthropogenic sources of Zn in the environment (air, water, soil) are related to mining and metallurgic operations involving Zn and use of commercial products containing Zn . Zn concentration was 196.94 ± 113.53 ppm in blue rock pigeon, 412.36± 270.88 ppm in eurasian collared dove, 229.77± 153.06 ppm in rose ringed parakeet, 721.81± 125.84 ppm in common myna, 358.61± 225.22 ppm in house crow, 464.97± 314.10 ppm in common babbler, 128.14± 8.06ppm in cattle egret, 473.99± 118.36 ppm in red wattled lapwing and 90.49 ppm in spotted owlet (Table 11, Fig. 13). Zn was lowest in excreta of cattle egret and it was its 2- fold in blue rock pigeon and rose ringed parakeet, 3- fold in eurasian collared dove and house crow, 4- fold in common babbler and red wattled lapwing and 6- fold i.e. highest in common myna. Several cases of Zn toxicosis have been seen in Hispaniolan Amazon parrots (*Amazona vertralis*) with liver zinc levels ranging from 110 to 359 µg/g wet weight (Lewis *et al* 2001). Carpenter *et al* (2004) reported that a trumpeter swan(*Cygnus buccinator*) that appeared to have died of Zn poisoning had 154mg/kg (154 µg/g)wet weight in liver. Likewise, Levengood *et al* (1999) and Sileo *et al* (2003) found signs of Zn poisoning in birds with liver concentrations as low as 473 and 280mg/kg (473 and 280 µg/ g)dry weight respectively. From these observations it can be concluded that level of Zn detected in excreta of 8 bird species indicated the high risk to these species. Bravo *et al* (2005) reported Zn concentration of 202.57 µg/ g in excreta of black vulture (*Coragyps atratus).*Weight loss under acute Zn toxicity has often been described (Levengood *et al* 1999). Takekawa *et al* (2002) found that total carcass mass decreased with increasing Zn concentrations in lesser (*Aythya affinis*) and greater scaup (*Aythya marila*). In captive birds, high doses of Zn could induce decreased food intake by way of decrease appetite and/or digestibility of prey, therefore explaining decreased body weight (Sundaresan *et al* 2008). Dewar *et al* (1983) experimentally poisoned chickens and reported lesions of the exocrine pancreas including dilation of acinar lumina, cytoplasmic vacuolation, cytoplasmic globule formation, necrosis, numerous mitotic figures and interparenchymal fibrosis. In an ultrastructure study of Zn toxicity in Pekin ducklings, Kazacos and Van Vleet (1989) reported apoptosis; attenuated, cuboidal, atrophic acinar cells;

interstitial fibrosis; and the formation of duct-like structures embedded in fibrous connective tissue. They found only minimal inflammatory response and the islets were normal. Similar lesions were reported in four species of captive diving ducks that had ingested pennies (Zdziarski *et al* 1994). However, Sileo *et al* (2003) reported the first case of Zn poisoning in free-ranging wild birds i.e. four waterfowl ((three *Branta canadensis*, one *Anas platyrhynchos*) in the Tri-State Mining District (Oklahoma, Kansas and Missouri, USA) had mild to severe degenerative abnormalities of the exocrine pancreas, as well as tissue (pancreas, liver).

4.5.9 Mangansese (Mn) in excreta of different bird species

Mn supports the immune system, regulates blood sugar levels and is involved in the production of energy and cell reproduction. Burning of diesel fuel is one of the sources of atmospheric Mn which is used as an anti-knocking agent in leaded gasoline (Qadir *et al* 2008). Mn concentration was 128.66 ± 32.87 ppm in blue rock pigeon, 625.12± 399.09 ppm in eurasian collared dove, 323.77± 181.20 ppm in rose ringed parakeet, 1075.83± 151.92 ppm in common myna, 221.78± 112.33 ppm in house crow, 401.43± 273.13 ppm in common babbler, 242.31± 51.60 ppm in cattle egret,760.41± 401.43 ppm in red wattled lapwing and 448.7 ppm in spotted owlet (Table 11, Fig. 14). Mn concentration was lowest in excreta of blue rock pigeon and it was its 2-fold in house crow and cattle egret, 3- fold in rose ringed parakeet and common babbler, 5- fold in eurasian collared dove, 6- fold in red wattled lapwing and 9- fold i.e. highest in common myna. Mn is regulated in birds primarily by excretion in the faeces, however hens also excrete Mn in eggs. Teratogenic effects (such as micromelia, twisted limbs, haemorrhage and neck defects), behavior impairments, altered growth rates and reduction of haemoglobin formation have been linked to sub lethal Mn exposure in animals and avian embryos (Burger and Gochfeld 1995). Mn tends to accumulate in bone, liver, pancreas and kidney of avian species. Presence of Mn concentrations in feathers could be coupled to concentrations in the contaminated ingesta and to some extent to air (Hui 2002), however, inhalation also provides small Mn exposure. Mn concentration was reported in feathers of cattle egret (26.9µg /g from Pakistan and 36.6 µg/ g from Hongkong). However, in present work concentration of Mn was much higher in excreta of 8 bird species as compared to concentration reported in feathers of cattle egret. No trophic level relationships have been found in a number of studies for Mn (Borga *et al* 2006) but Horai *et al* (2007) suggested the distribution of Mn in body of birds affected by diet.

In the present studies among the different bird species studied due to less amount of excreta of spotted owlet only one reading was taken for analysis and that could not depict the actual levels of heavy metals in this species, thus no interspecific comparison was made with this species. The blue rock pigeon excreted lowest amount of As, Cd, Cr, Fe, Ni and Mn and cattle egret excreted lowest amount of Pb, Cu and Zn in the faeces. However, common myna excreted highest amount of As, Cr, Fe, Ni, Zn and Mn, red wattled lapwing excreted highest amount of Cd and Pb and common babbler excreted highest amount of Cu. This variation in levels of excreted metal concentrations may be attributed to different feeding behaviours of different species . Trophic-level relationships have been reported for a range of species and for a number of contaminants (Burger 1993, Lemly 1993, Sundlof *et al* 1994, Barron 1995). In general, species that are higher on the food chain accumulate higher levels of contaminants. It has been demonstrated that plant consuming species accumulated fewer metals than species feeding at higher trophic positions on food chain (Eens *et al* 1999, Burger and Gochfeld 2000a, Goutner *et al* 2001). Secondly, it may be due to physiological differences between the different species as metabolic rates of small passerines vary inversely with body weight and directly with activities such as flight and rest (Teal 1969, Welty 1975). There were significant interspecific differences in all metal levels (arsenic, cadmium, chromium, lead, manganese, mercury and selenium) in feathers of bald eagle, common eider, glaucous-winged gull, pigeon guillemot and tufted puffin from the Aleutian Chain of Alaska (Burger and Gochfeld 2009).

The different bird species were observed to follow particular order for the excretion of different heavy metals in present studies. The blue rock pigeon follows the order Fe> Zn> Mn> Cu > Pb > Ni > Cr > As > Cd for excretion of heavy metals in faeces, Eurasian collared dove follows Fe> Mn> Zn> Cu > Cr > Pb > Ni > As > Cd, rose ringed parakeet follows Fe> Mn> Zn> Cu > Pb > Cr > Ni > Cd >As, common myna follows Fe> Mn> Zn> Pb > Cr > Cu > Ni > Cd> As, house crow follows Fe> Zn> Mn> Pb > Cu > Ni > Cr > Cd> As, common babbler follows Fe> Zn> Mn> Cu > Cr > Pb >Ni > As> Cd, cattle egret follows Fe> Mn> Zn> Cr > Cu> Ni > Pb > As >Cd and red wattled follows Fe> Pb > Mn> Zn> Cu > Cr > Cd> Ni > As. Thus in all bird species, maximum excretion was of Fe in their faeces and minimum of either As or Cd which reveals the possible retention of these toxic heavy metals affecting these bird species in a chronic manner by increasing reproductive dysfunctions/ endocrine disruption.

There exists a wide range of each heavy metal in every bird species studied. Range of As varies from 0.50 to 1.27 ppm in blue rock pigeon, 1.29 to 16.60 ppm in eurasian collared dove, 1.25 to 2.00 ppm in rose ringed parakeet, 0.83 to 14.18 ppm in common myna, 0.49 to 1.50 ppm in house crow, 0.36 to 13.76 ppm in common babbler, 1.75 to 3.36 ppm in cattle egret and 0.417 to 20.85 ppm in red wattled lapwing (Table 12). The Cd range varies from 0.03 to 0.75 ppm in blue rock pigeon, 0.26 to 7.50 ppm in eurasian collared dove, 0.09 to 4.17 ppm in rose ringed parakeet, 6.67 to 12.51 ppm in common myna, 0.03 to 10.75 ppm in house crow, 0.01 to 5.42 ppm in common babbler, 0.59 to 4.25 ppm in cattle egret and 3.34 to 82.57 ppm in red wattled lapwing . The range of Pb was from 7.63 to 20.00 ppm in blue rock pigeon, 3.90 to 95.08 ppm in eurasian collared dove, 0.50 to 67.14 ppm in rose ringed parakeet, 47.00 to 240.19 ppm in common myna, 2.42 to 169.25 ppm in house crow, 3.40 to 54.21 ppm in common babbler, 6.50 to 7.97 ppm in cattle egret and 52.55 to 1680.93 ppm in red wattled lapwing . The Fe range varies from 430- 1793 ppm in blue rock pigeon, 3554 to 52079 ppm in eurasian collared dove, 716 to 17005 ppm in rose ringed parakeet, 4824 to 50119 ppm in common myna, 1673 to 3726 ppm in house crow, 308 to 29748 ppm in common babbler, 8102 to 19022 ppm in cattle egret and 10971 to 63713 ppm in red wattled lapwing. The Cr range varies from 3.13 to 3.50 ppm in blue rock pigeon, 4.84 to 156.00 ppm in eurasian collared dove, 2.32 to 57.55 ppm in rose ringed parakeet, 29.19 to 171.80 ppm in common myna, 1.29 to 16.75 ppm in house crow, 0.20 to 98.83 ppm in common babbler, 7.98 to 48.75 ppm in cattle egret and 41.70 to 163.05 ppm in red wattled lapwing . The Cu range varies from 9.74 to 75.75 ppm in blue rock pigeon, 9.80 to 235 ppm in eurasian collared dove, 11.69 to 68.81 ppm in rose ringed parakeet, 40.45 to 114.68 ppm in common myna, 9.91 to 110.5 ppm in house crow, 30.44 to 127.60 ppm in common babbler, 15.48 to 24.75 ppm in cattle egret and 41.70 to 163.05 ppm in red wattled lapwing . The Ni range varies from 6.26 to 12.25 ppm in blue rock pigeon, 8.63 to 80.64 ppm in eurasian collared dove, 5.64 to 43.79 ppm in rose ringed parakeet, 22.94 to 82.15 ppm in common myna, 0.21 to 29.25 ppm in house crow, 1.07 to 52.96 ppm in common babbler, 4.80 to 20.5 ppm in cattle egret and 21.27 to 77.15 ppm in red wattled lapwing . The Zn range varies from 54.49 to 475 ppm in blue rock pigeon, 80.39 to 1075.86 ppm in eurasian collared dove, 13.35 to 446.19 ppm in rose ringed parakeet, 562.95 to 1029.99 ppm in common myna, 67.94 to 910 ppm in house crow, 79.19 to 1234.32 ppm in common babbler, 115.09 to 147.50 ppm in cattle egret and 271.05 to 754.77 ppm in red wattled lapwing .The Mn range varies from 81.49 to 208.75 ppm in blue rock pigeon, 123.74 to 1602.53 ppm in eurasian collared dove, 9.94 to 637.59 ppm in rose ringed parakeet, 830.25 to 1440.74 ppm in common myna, 52.44 to 494.25 ppm in house crow, 59.74 to 1069.61 ppm in common babbler, 130.94 to 349.75 ppm in cattle egret and 362. 79 to 1311.88 ppm in red wattled lapwing. Lucia *et al* (2010) reported As was influenced by sex. Female birds displayed higher concentrations in liver and feathers than did male birds. Taggart *et al* (2006) also observed that for all species studied, sex was a significant factor with respect to liver As. Age was one of the main factors affecting Cd accumulation, as observed in waterfowl from southern Spain (Gomez *et al* 2004). Adult birds displayed higher concentrations of Cd compared with juvenile birds (Lucia *et al* 2010). Lucia *et al* (2010) reported that feathers were more contaminated with Pb in juveniles as compared with the adult birds. Decreased Pb concentrations in feathers of adult birds can be explained by the achievement of moulting in adults and this mechanism enables the excretion and removal of metals. However, in the present observations as the excreta was collected from the fields and it included birds of different age groups as well as sexes

Table 12: Concentration range of heavy metals (ppm) in excreta of different birds.

S.No.	Bird Species	Arsenic	Cadmium	Lead	Iron	Chromium	Copper	Nickel	Zinc	Manganese
1.	Blue rock pigeon	0.50 – 1.27	0.03 – 0.75	7.63 – 20.00	430.00- 1793.65	3.13 – 3.50	9.74 – 75.75	6.26 – 12.25	54.49 – 475.00	81.49 - 208.75
2.	Eurasian collared dove	1.29 – 16.60	0.26 – 7.50	3.90 – 95.08	3554.65- 52079.13	4.84 – 156.00	9.80 – 235.00	8.63- 80.64	80.39 – 1075.86	123.74 - 1602.53
3.	Rose ringed parakeet	1.25 – 2.00	0.09 – 4.17	0.50 – 67.14	716.12- 17005.26	2.32- 57.55	11.69- 68.81	5.64- 43.79	13.35 – 446.19	9.94 - 637.59
4.	Common myna	0.83 – 14.18	6.67 – 12.51	47.00 – 240.19	4824.69 – 50119.23	29.19 – 171.80	40.45 – 114.68	22.94 – 82.15	562.95 – 1029.99	830.25 – 1440.74
5.	House crow	0.49 – 1.50	0.03 – 10.75	2.42 – 169.25	1673.65 – 3726.65	1.29 – 16.75	9.91 – 110.5	0.21 – 29.25	67.94 - 910	52.44 – 494.25
6.	Common babbler	0.36 – 13.76	0.01 – 5.42	3.40 – 54.21	308.35 – 29748.78	0.20 – 98.83	12.85- 421.59	1.07- 52.96	79.19 – 1234.32	59.74 – 1069.61
7.	Cattle egret	1.75 – 3.36	0.59 – 4.25	6.50 – 7.97	8102.15 – 19022.50	7.98 – 48.75	15.48 – 24.75	4.80 – 20.50	115.09 – 147.50	130.94 – 349.75
8.	Red wattled lapwing	0.417 – 20.85	3.34 – 82.57	52.55 – 1680.93	10971.27 - 63713.43	41.70 – 163.05	30.44 – 127.60	21.27 – 77.15	271.05 – 754.77	362.79 – 1311.88

The data is presented as range of concentration of heavy metals observed in 2 or 3 samples different birds of pooled excreta.

which may be responsible for variation in the concentration of As, Cd, Pb, Fe, Cr, Cu, Ni, Zn, Mn in these species.

The variation in different metal concentration in excreta of eight species of birds may be because of number of factors like as mentioned above like variation in age, size and sex of different birds from which excreta was collected. As compared to the concentrations of heavy metals in the body tissues of some of the bird species the levels of As, Cd, Pb, Cr, Cu, Ni, Zn and Mn were found to be in already reported toxic range. This reveals that all these birds get heavy metal contaminations from their environment. Further the present studies have revealed that most of the metals including As, Cd, Fe, Cr, Ni and Mn (Table 11) excreted in the faeces of blue rock pigeon were lowest as compared to other species and they were below the toxic levels as reported in body tissues of some birds. However, in common myna the level of toxic heavy metals was higher as compared to other species and was of level of toxic range of body tissues of other birds which may thus be a protective mechanism for its survival as this has been found to be a dominant species in the agrifields of PAU (Ishlash 2010).

These present observations and earlier reports revealed that the chronic exposure of birds to potential toxic metals insufficient to produce outright mortality or other acute effects may lead to profound consequences on birds such as decreased reproductive function, increased suspectibilty to diseases or other stresses (Furness 1996) and changes in behavioral patterns. These observations further throw light that significant fluctuations in contaminated levels are present in the environment where these birds live (Erwin and Custer 2000).

4.6 Levels of pesticide residue metabolites in bird excreta

The pesticide residue levels were analyzed in 5 grams samples of excreta of different bird species which included pooled samples of about 30- 50 pellets of each species.

4.6.1 Organophosphates (OPs) residues in excreta of different bird species

The residues of different organophosphates (OPs) dichlorvos, acephate, phorate, monocrotophos, dimethoate diazinon, chlorpyriphos methyl, methyl parathion, fenitrothion, malathion, chlorpyriphos, chlorphenvinphos, quinalphos, profenophos, ethion, triazophos, edifenophos,anilophos, phosalone were analyzed in dry excreta of blue rock pigeon, eurasian collared dove, common myna, house crow, common babbler, cattle egret and red wattled lapwing (Table 13, Plate VIII, Plate IX). It was observed that all these pesticides were non-detecbale in 5 gram excreta of all these bird species .The only pesticide detected was chlorpyriphos which was found to be in concentration of 0.046 ppm in house crow and 0.33 ppm in cattle egret (Fig. 15, Plate IX a and c). OPs are most commonly used pesticides throughout the world because of their low bioaccumulation properties in comparison to OCs. Since the early 1980s, both OPs and carbamates (CMs) have been used as pesticide. Both these insecticides inhibit acetylcholinestrase (AChE) at the postsynaptic membrane of cholinergic synapses (Bishop *et al* 1998) in the central and peripheral nervous system of all vertebrate species. Birds appear to be more sensitive to acute exposure to anticholinestrase pesticides due to reduced levels of acetylcholinestrase detoxifying enzymes (Parker and Goldstein 2000). Due to high activity of AChE in the brain of birds (Westlake *et al* 1983), the rate of binding to OPs and CMs are more rapid than other vertebrates (Hill 1992).

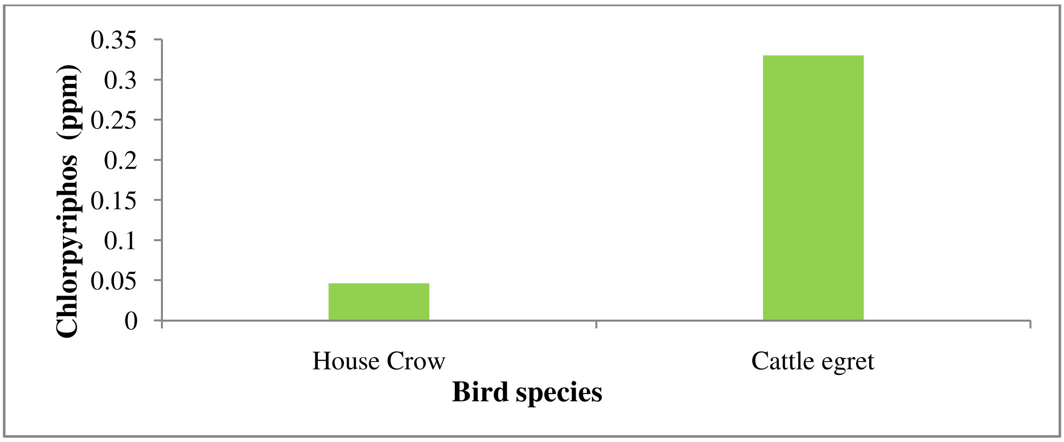


Fig. 15: Concentration of Chlorpyriphos in excreta samples

Chlorpyriphos, an OP, inhibits AChE in a way that has cross generational implications (Anway *et al* 2005) and it severely affects birds (Mitra *et al* 2011). Thus it corroborates with our observations in house crow and cattle egret. Moreover, chlorpyriphos is reported to be commonly used in the insecticidal formulations for control of ticks in cattle and buffaloes at dairy form of GADVASU from where the excreta of cattle egret mainly and to some extent of house crows were collected. Thus it throws light on a direct correlation between the use of chlorpyriphos and its entry into these birds through oral as well as dermal route by consumption of ticks while sitting on the cows and buffaloes.

4.6.2 Organochlorines (OCs) residues in excreta of different bird species

The organochlorines (OCs) *viz.* α-HCH, γ –HCH, β-HCH, heptachor, δ-HCH, aldrin, dicofol, op DDE, α- endosulphan, DDE, DDD, DDT, β-endosulphan, pp DDD, pp DDT and endosulphan sulphate pesticide residues were analysed for their occurrence in the dry excreta of all the bird species included in the present studies (blue rock pigeon, eurasian collared dove, common myna, house crow, common babbler, cattle egret and red wattled lapwing) (Plate X, Plate XI). There was no OC residue in the excreta of any of these species which may be because of their undetectable levels in 5 gram samples of bird excreta or these may not be present at all in excreted feces of these birds. In India, 145 pesticides are registered for use at present. Pesticide production began in 1952 in India. During the DDT era about 85% of farmers of India used OCs pesticides at rate of 0.39 kg/ha covering 282 million hactares of agricultural land (NCAER 1967). OCs are no longer in use in several countries, but some of them like aldrin , dieldrin, lindane and endosulphan are still in use in developing countries. In India the consumption of pesticides is highest in Andhrapradesh (33%), followed

Table 13: Organophosphates residue in excreta of different bird species

S.No	Bird Species	Dichlorvos	Acephate	Phorate/ Monocrotophos	Dimethoate	Diazinon	Chlorpyriphos methyl/ Methyl parathion	Fenitrothion	Malathion	Chlorpyriphos	Chlorphenvinphos	Quinalphos	Profenophos	Ethion	Triazophos	Edifenophos	Anilophos	Phosalone
1.	Blue rock pigeon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	Eurasian collared dove	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	Common myna	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	House crow	-	-	-	-	-	-	-	-	0.046 ppm	-	-	-	-	-	-	-	-
5.	Common babbler	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6.	Cattle egret	-	-	-	-	-	-	-	-	0.33 ppm	-	-	-	-	-	-	-	-
7.	Red wattled lapwing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

by Punjab, Karnataka, tamilnadu, Maharastra, Haryana, Gujrat, Uttar Pradesh and the remaining states account less than 9.5% of the total by Singhal (1969). However, this amount of pesticide use remained almost unchanged during last five decades (Vyas 1998). In India, pesticide use has increased dramatically and now it is becoming a global problem.

4.6.3 Synthetic pyretheroids residues in excreta of different bird species

The residues of synthetic pyretheroids; bifenthrin, fenpropathrin, γ- cyhalothyrin, β-cyfluthrin, cypermethrin, fluvalinate, fenvalrate and deltamethrin were also analysed and found to be absent in 5 gram excreta of different bird species i.e. blue rock pigeon, eurasian collared dove , common myna, house crow, common babbler, cattle egret and red wattled lapwing) (Plate X and XI).

Pesticides are ubiquitous contaminants of our environment and have been found in soil, air, water, human and animal tissues in samples from all over the world. Injuries to population of birds from environmental pollutants and pesticides are obvious indicators of environmental damage. Thousands of birds died each year because of pesticide use in the past. But the bird kills are hard to document due to many reasons. However, pesticides have most striking effects on birds particularly on carnivorous species which remains on higher trophic level in food chains such as bald eagles, hawks and owls. These birds are often rare, endangered and suspectible to pesticide residues through food chain. Insect eating birds such as patridges, growls and pheasants have decreased due to loss or decrease in insect population in agricultural fields through insecticides. Thus the pesticide effects on birds though are multiple but they yet remain poorly understood. However, decline in bird population in many parts of the world is a matter of serious concern. The pesticide induced death in birds is difficult to estimate accurately as birds may die away from site of poisoning or their carcasses decompose quickly or may be eaten by the scavengers; as a result small portions of such deaths are documented. In USA one of the three bird species is either endangered or threatened or is in need of conservation (NABCI 2009). The decline is highest in grasslands and wetland birds (Rich *et al* 2004). On an average population of all common birds and forest birds have declined by about 10% in Europe between 1980 and 2006 and in farmland bird population, this is about 48% (EBCC 2008). The reduction of grassland habitas for agriculture affects the birds detrimentally. For 187 globally threatened avian species, the primary pressure on survival is chemical pollution including pesticides. In USA some 50 pesticides are known to kill songbirds, gamebirds,raptors, seabirds and shorebirds (BLI 2004).

Some of the most troublesome pesticides are DDT, dieldrin, diazinon, parathion, aldicarb, atrazine, paraquat and glyphosate. However the state of Punjab in India is one of the highest user of chemical pesticides after the unshering of green revolution. The pesticide consumption trend in India has increased from 2353 MT in 1955 to 40672 MT in 2005 . In

PLATE VIII: Chromatograms for pesticide (OPs) residue analysis in excreta of different species of birds

a. **Chromatogram of standard solutions of OPs**
b. **Chromatogram for OPs residue analysis in blue rock pigeon**
c. **Chromatogram for OPs residue analysis in eurasian collared dove**
d. **Chromatogram for OPs residue analysis in common myna**

PLATE VIII

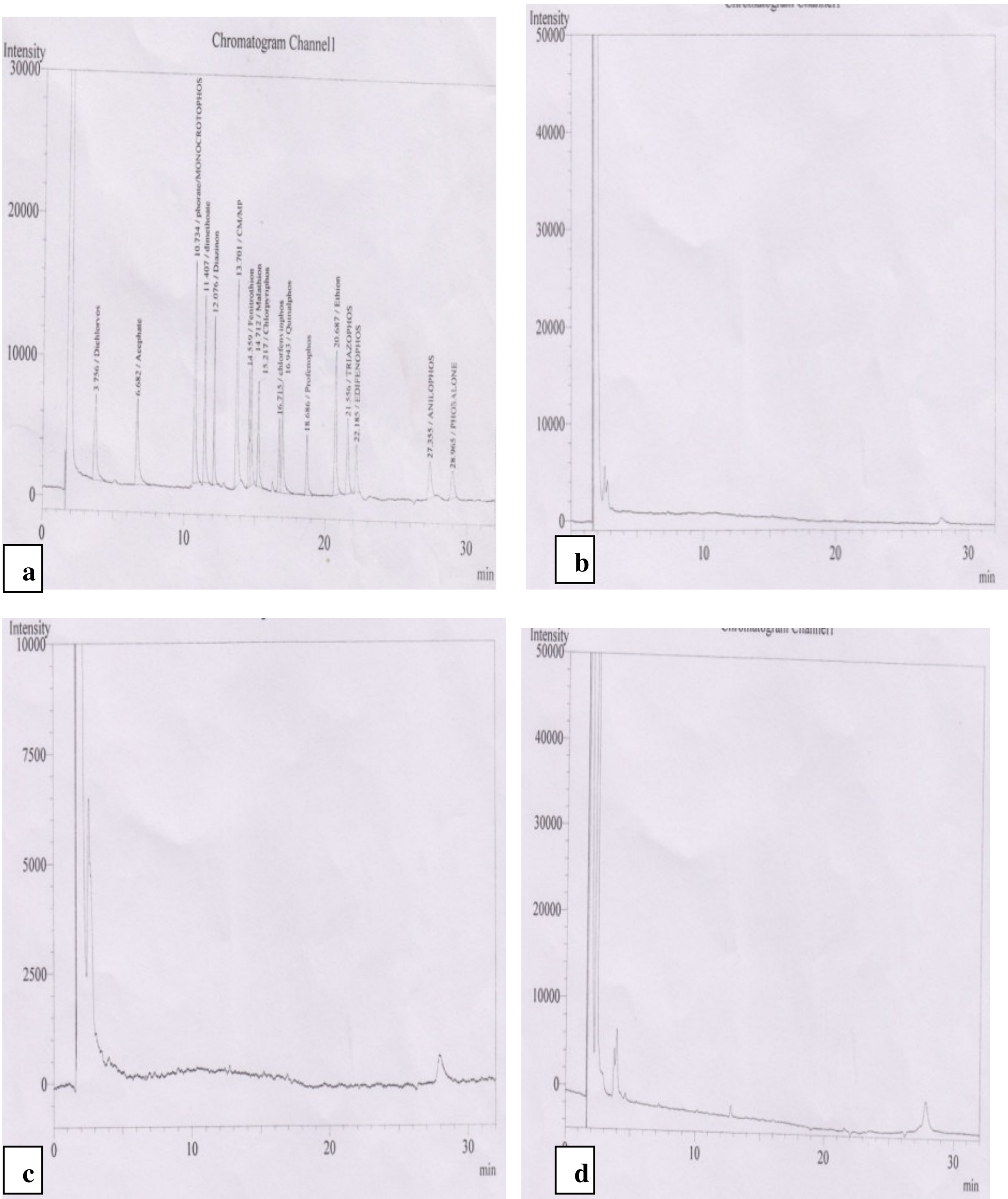

Chromatogram Channel1
Intensity
min
3.756 / Dichlorvos
6.682 / Acephate
10.734 / phorate/MONOCROTOPHOS
11.407 / dimethoate
12.076 / Diazinon
13.701 / CM/MP
14.559 / Fenitrothion
14.712 / Malathion
15.217 / Chlorpyriphos
16.715 / chlorfenvinphos
16.943 / Quinalphos
18.686 / Profenophos
20.687 / Ethion
21.556 / TRIAZOPHOS
22.185 / EDIFENOPHOS
27.355 / ANILOPHOS
28.965 / PHOSALONE
a
b
c
d

PLATE IX: Chromatograms for pesticide (OPs) residue analysis in excreta of different species of birds

a. **Chromatogram for OPs residue analysis in house crow**
b. **Chromatogram for OPs residue analysis in common babbler**
c. **Chromatogram for OPs residue analysis in cattle egret**
d. **Chromatogram for OPs residue analysis in red wattled lapwing**

PLATE IX

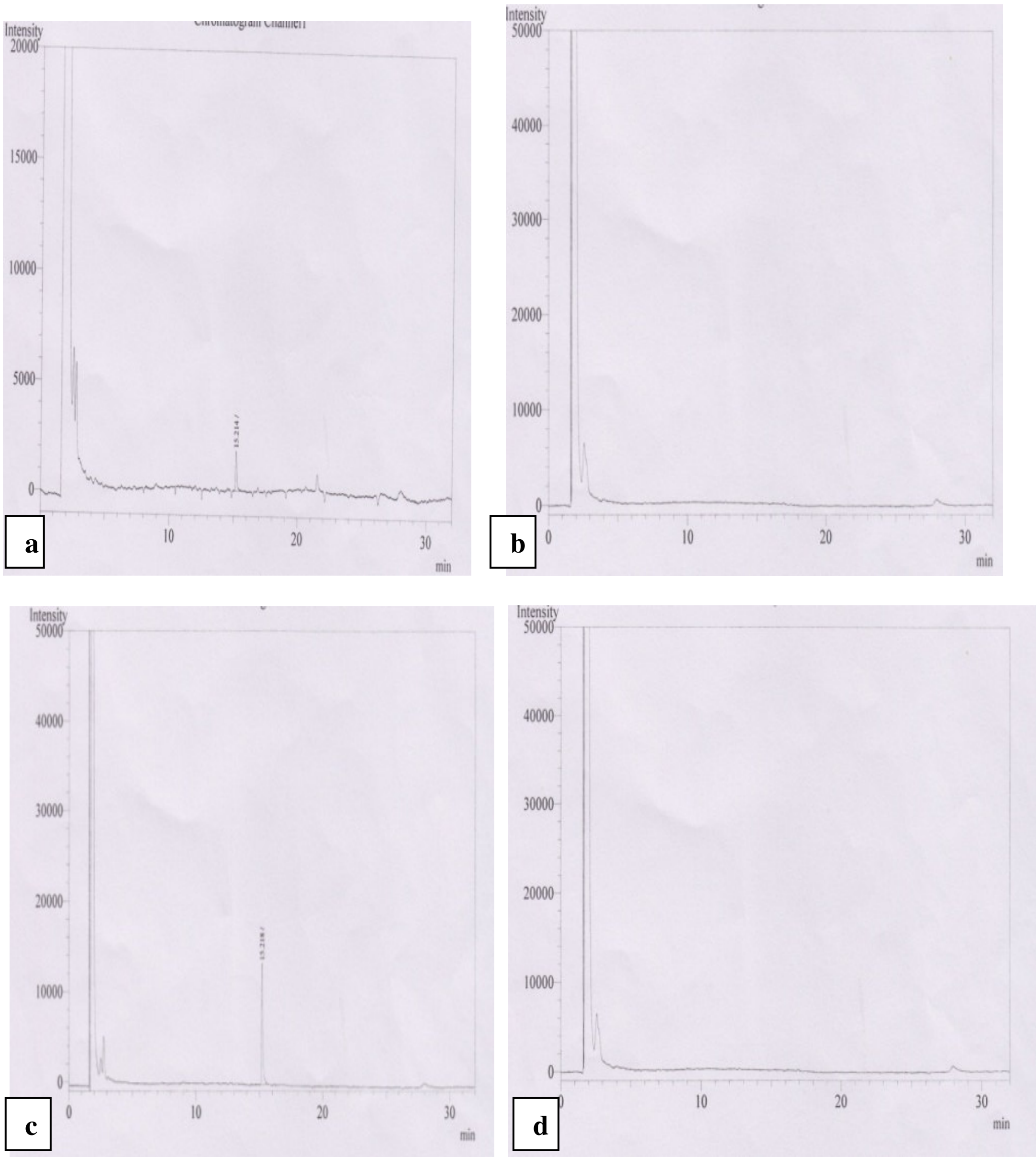


PLATE X: Chromatograms for pesticide (OCs + synthetic pyretheroids) residue analysis in excreta of different species of birds

a. **Chromatogram of standard solutions of OCs+ Synthetic pyretheroids**
b. **Chromatogram for OCs+ Synthetic pyretheroids residue analysis in blue rock pigeon**
c. **Chromatogram for OCs+ Synthetic pyretheroids residue analysis in eurasian collared dove**
d. **Chromatogram for OCs+ Synthetic pyretheroids residue analysis in common myna**

PLATE X

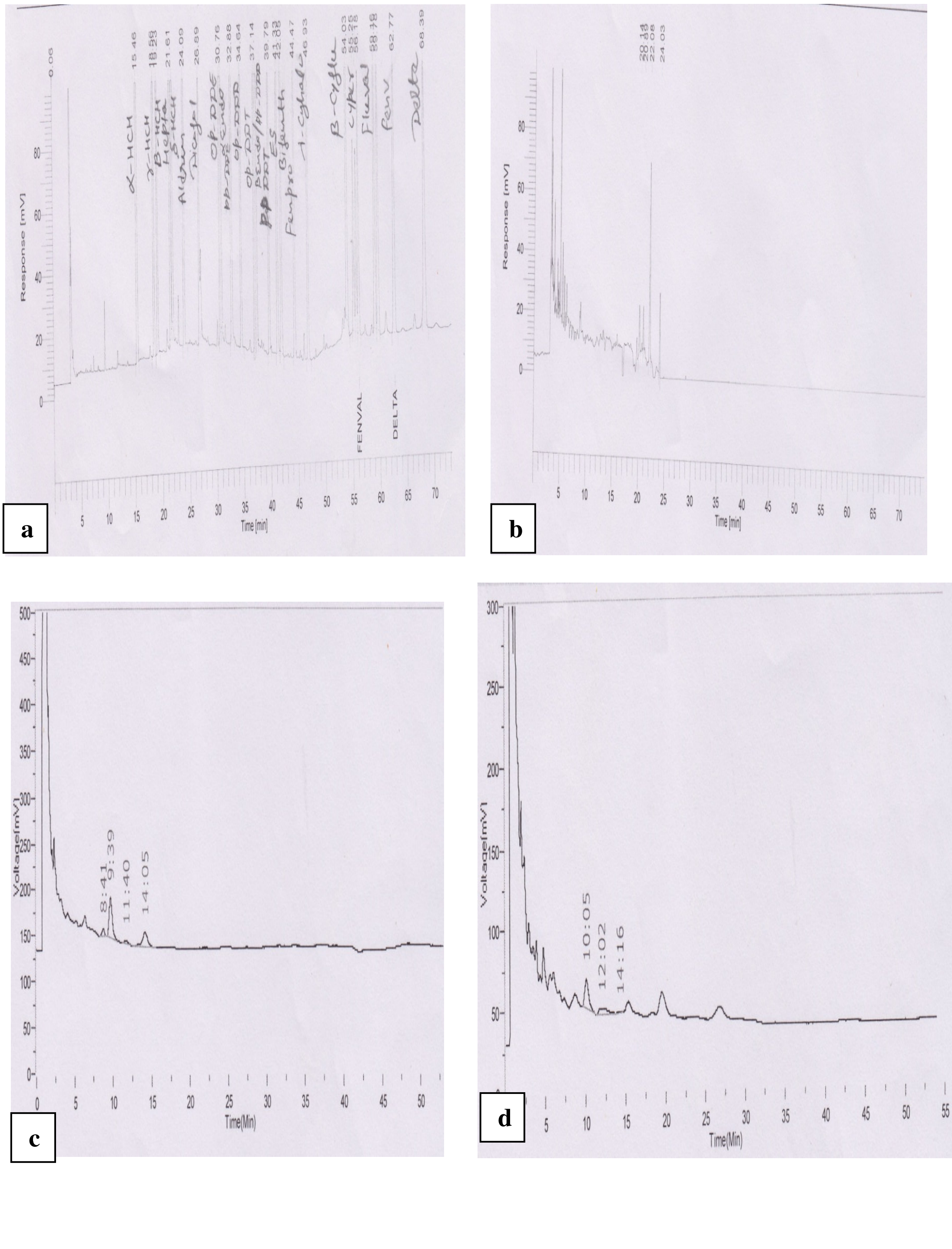
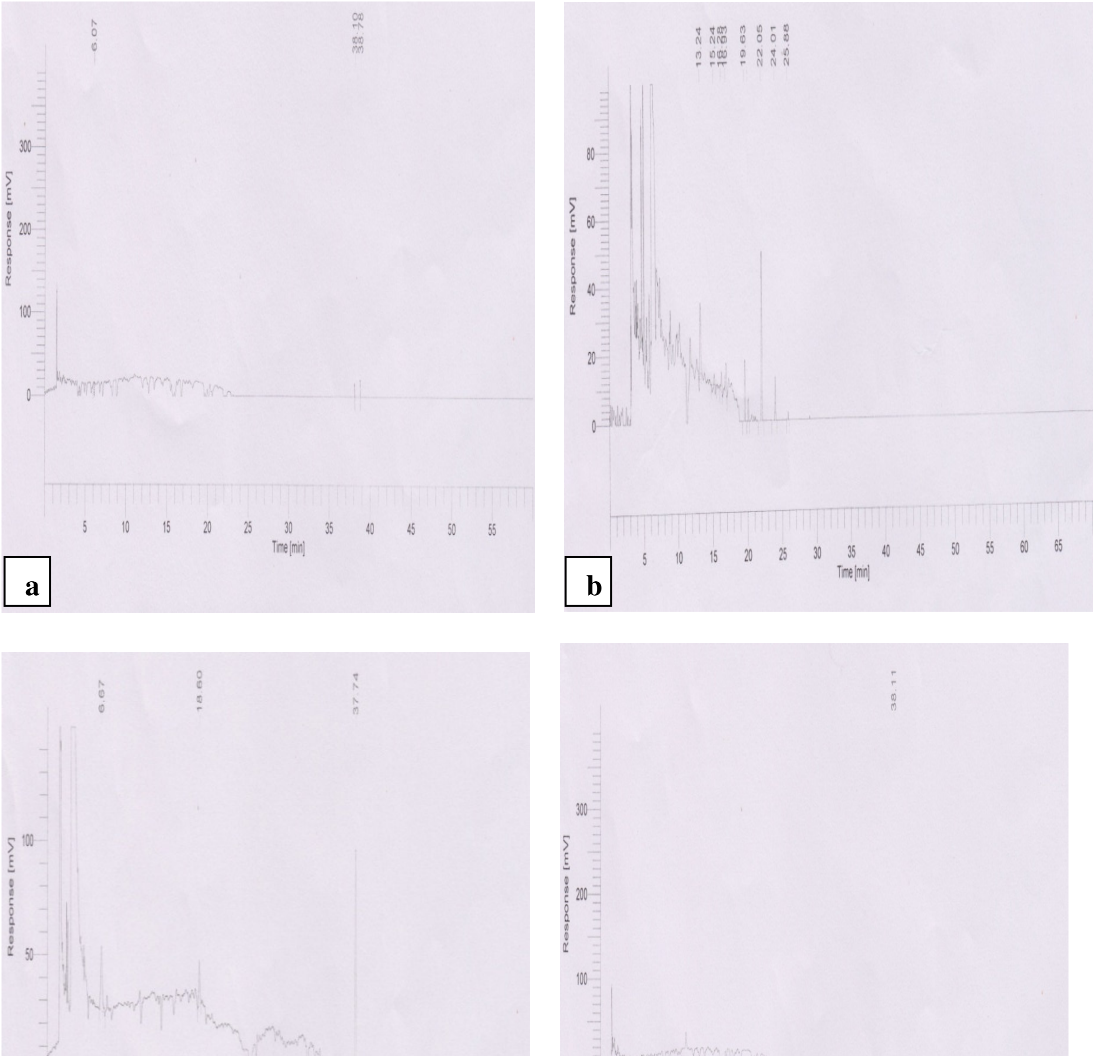

a
Response [mV]
Time [min]
FENVAL
DELTA
b
Response [mV]
Time [min]
c
Voltage(mV)
Time(Min)
8:41
9:39
11:40
14:05
d
Voltage(mV)
Time(Min)
10:05
12:02
14:16

PLATE XI: Chromatograms for pesticide (OCs + synthetic pyretheroids) residue analysis in excreta of different species of birds

a. **Chromatogram for OCs+ Synthetic pyretheroids residue analysis in house crow**
b. **Chromatogram for OCs+ Synthetic pyretheroids residue analysis in common babbler**
c. **Chromatogram for OCs+ Synthetic pyretheroids residue analysis in cattle egret**
d. **Chromatogram for OCs+ Synthetic pyretheroids residue analysis in red wattled lapwing**

PLATE XI


a
Response [mV]
Time [min]
6.07
38.10
38.78
b
Response [mV]
Time [min]
13.24
15.24
19.63
22.05
24.01
25.88
c
Response [mV]
Time [min]
6.67
18.60
37.74
d
Response [mV]
Time [min]
38.11

Punjab this has increased from 3200 Metric tons (MT) in 1980-81 to 7300 MT in 1994-95 but came down to 5970 MT in the year 2005-06 . Currently, the state consumes about 17 percent of total pesticides used in India. In a recent survey for detection of pesticides / insecticides in the blood samples of human beings in four different villages of Punjab in Bathinda and Ropar districts, the residues of OCs were detected in most of the samples as they are persistent in nature due to their slow decomposition rate, long half life and high stability in the environment inspite of the fact that many of the OCs are banned for their use in India and most of them are replaced by less persistent pesticides i.e. OPs. Out of the 14 OPs analysed in whole blood samples. 4 of them depicted to be monotrophos, chlorpyriphos, malathion and phosphamidon were most commonly detected. Chlorpyriphos is reported to be the 4th highest consumed pesticide in India and was detected in 85% of blood samples.

Many pesticides which donot remain at a source but travel long distances are called transboundary pollutants. Persistent Organic Pollutants (POPs) like DDT can travel far from their source, tend to accumulate in the fatty tissue of organisms and increase in concentration as they move up the food chain. The OC insecticides block insect nervous system causing malfunction, tremors and deaths. During travelling a long distance, many birds get exposed to pollutants and pesticides. As a lot of damage was caused by DDT to wildlife and human (Rattner 2009), studies for its residues in bird tissues and field trials showed that DDT was more toxic to aquatic than to its terrestrial vertebrates (Mitchell *et al* 1953). In 1962 some restrictions were imposed on use of cyclodiene pesticides like aldrin and dieldrin. After the release of book 'Silent Spring' many pesticidal effects such as bioaccumulation, resistance, extensive intensification of freshwater and ecological imbalances were addressed (Carson 1962). Other reports like problem of reproductive dysfunction, egg shell thinning, metabolic changes, deformities and birth defects, cancer, behavioral changes, abnormal thyroid activities, endocrine disruption, immunosuppression, feminization of males and masculinization of females (Orris *et al* 2000) were reported in USA in predatory birds like eagles, cormorants inhabiting the heavily polluted great lake due to POPs. Gradually the OCs were replaced by OPs and carbamate pesticides which were less toxic than chlorinated hydrocarbons. In the next decades studies on clinical effects of pesticides like embryo-toxicity, immune-toxicity, reproductive effects, histopathology, endocrine functions, serum enzymes and cellular damage, oxidative stress, teratogenicity and biochemical indicators were examined (Walker 2003). Besides direct toxicity insecticides, herbicides and agricultural practices were found to exert ecological effects to wildlife by altering habitat, vegetation, insect prey base and other parameters.

Thus the observations on pesticide residue in the excreta of bird species of agrifields, revealing the detection of only one type of OP i. e. chlorpyriphos in present studies may be because of more use of this pesticide in the cropland areas of Punjab as most of the pollutants can travel long distances thus act as transboundary pollutants. They caused widespread population decline of raptorial birds like the peregrine falcon, the sparrow hawk and bald eagle. OP and CM insecticides donot bioaccumulate. The birds have also been shown to be more suspectible than mammals to many pesticides because of their relatively inefficient system of detoxification (Walker 1978). The high toxicity of OPs to birds, such as diazinon (Machin *et al* 1976) and pirimiphosmethyl (Brealey *et al* 1980) has been correlated with relatively low 'A' esterase activity in avian plasma. The low activity of certain detoxifying enzymes in birds may result in the efficient accumulation of certain pollutants that are slowly metabolized and are present at low levels in their food. The understanding of the relationship between metabolism of pesticides and toxic action in birds is very limited. There is little information on the *in vivo* metabolism of pesticides and the relative importance of urine and bile as routes of excretion for these compounds. Even the present studies on pesticide residues in bird excreta donot reveal any direct indications of the impacts of these chemicals as environment pollutants on bird populations except for chlorpyriphos in cattle egret and house crow. Chipman and Walker (1981) have reported that metabolism and excretion of HCE is slower in the pigeon than in the male rat, thus providing further evidence for the limited capability of the species to perform certain detoxifying transformations and thus making it undectable in faecal matter.

The non-detecability of all the OCs and synthetic pyretheroids in excreta of all seven bird species studied in present work may be because of their less use for insect pest control in Punjab as the pesticides like aldrin, benzene hexachloride, calcium cyanide, chlordane, copper acetarsenite, bromochloropropane, endrin, ethyl mercury chloride, ethyl parathion, heptachlor, menazone, nitrofen, paraquat dimethylsulphate, pentachloronitrobenzene, pentachlorophenol, phenylmercury acetate, sodium methane arsenate, tetradfon, toxafen, aldicarb, chlorobenzilate, dieldrin, maleic hydrazide, ethyl dibromide TCA, DDT, dieldrin, diazinon, parathion, aldicarb, atrazine, paraquat and glyphosate are banned in India. Moreover, the US Environmental Protection Agency conducts risk assessments of insecticide applications to wild birds using a model that is limited to the dietary route of exposure. However, free-flying birds are also exposed to insecticides via the inhalation and dermal routes (Vyas *et al* 2007). Birds may be exposed via inhalation during an application and following an application as the insecticide volatilizes or from inhaling contaminated airborne particles. The oral routes includes ingestion of contaminated food and water and ingestion of the insecticide while preening contaminated feathers. The dermal exposure route involves absorption of the insecticide via eyes, skin, and feet. Preening may also facilitate dermal absorption by spreading the insecticide over the skin (Driver *et al* 1991). Therefore it can be concluded that because of the multiple routes of exposure of the birds to chemical pollutants and their variable metabolic potentials, the excreta may not be a very good monitoring tool for correlating the pesticide exposure and its effects on bird population decline. The use of hair, a keratinous tissue, has recently been evaluated as a method for the analysis of persistent organic pollutants (POPs) (Zupancic-Kralj *et al* 1992, Dauberschmidt and Wennig 1998, Covaci *et al* 2002, Altshul *et al* 2004, D'Have *et al* 2005). Because feathers are also composed of a keratinous matrix, they are potentially useful to study contamination with POPs. In contrast to hair, which is continuously growing, feathers grow only for a certain time and are connected to the bloodstream (and its circulating pollutants) only during this limited period (Jaspers *et al* 2004, 2006). Whereas many biomonitoring studies on organic pollutants have previously focused on bird eggs, feathers have the advantage that they can be collected irrespective of season, age, or gender (Janssens *et al* 2001). Moreover, because one feather can easily be removed from a living bird without causing severe damage, this technique could be valuable as a nondestructive biomonitoring tool for endangered species. The use of feathers as monitors of organic pollutants has only recently been investigated (Dauwe *et al* 2005, Jaspers *et al* 2006, 2007a, 2007b, Van den Steen *et al* 2007). However, the study by Behrooz *et al* (2009) on pesticides and Polychlorinated biphenyls (PCBs) in tail feathers was on 35 resident and migratory birds of southwest Iran kept in museum collections. There are no reports on the pesticide residue analysis in feathers of wild birds in natural habitats.

The initial heavy use of OC pesticides like DDT (considered to be POPs and later replaced by less persistent OPs and CMs) it seems that the bird population was tremendously affected initially by their bioaccumulation of OCs. The initial impacts were by direct induced killings of birds using lethal doses of pesticides(OCs). The second kind of effects were because of bioaccumulation of pesticides like DDT and DDE which resulted in reproductive dysfunctions, egg shell thinning, metabolic changes, birth defects and endocrine disruption and thus both the acute and chronic exposure to all kinds of pesticides caused increased mortality while sublethal levels adversely affect their reproductive functions and thus caused a significant decline in bird diversity/ population world over.

Chapter V

SUMMARY

Birds constitute an important component of agroecosystems. Agricultural fields provide a concentrated and highly predictable source of food to birds. Bird community in agricultural lands is characterized by overdominance of only a few grainivorous and omnivorous species while the rest of species are represented in very small numbers, some of which are rare. Avian management includes both conservation of useful species and control of pest birds. Birds provide an early warning of environmental problems. Due to heavy and indiscriminate use of chemical pesticides all over the world, the birds must have been contaminated with their residues and this has resulted in population decline of a number of bird species.The assessment of environmental impact of various pest control techniques on bird populations is also an essential component of management. There is great paucity of information on the pesticide effects on birds, which are multiple, but poorly understood. According to Wildlife Protection Act 1972, the capturing and killing of birds is legally banned by Govt. of India, therefore any analytical studies on the tissues of these animals are beyond the reach of scientists working in this area. Thus, the excreta of birds is the only source, which if analyzed can give an assessment of the harmful impact of environmental contaminants on these animals. In the present studies, the biological (microbial fauna; moulds, yeasts and bacteria) and chemical composition (heavy metals and pesticide residues) of the excreta of common birds with different feeding habits was analysed.

Feeding habits of common birds i.e. *Columba livia* (blue rock pigeon), *Streptopelia decaocto* (eurasian collared dove), *Psittacula krameri* (rose ringed parakeet), *Acridotheres tristis* (common myna), *Corvus splendens* (house crow), *Turdoides caudatus* (common babbler), *Bubulcus ibis* (cattle egret), *Vanellus indicus* (red wattled lapwing) and *Milvus Migrans* (pariah kite) were observed randomly and excreta collected in different agrifields and orchards of PAU, dairy farm of GADVASU as well as in residences of PAU and the Ludhiana city. It was observed that blue rock pigeon feeds voraciously in flocks and its food consists of dried chapatis, dalia, soozi, wheat flour, grains of wheat, rice and bajra and siliqua (fruit of *Brassica*). Eurasian collared dove feeds on wheat, rice, bajra grains and siliqua alongwith flocks of blue rock pigeon either solitary or in pairs. Both of these species are grainivorous. Rose ringed parakeet being frugivorous feeds in flocks on seeds of *Brassica*, corn, bajra, mango, guava and fruits. The common myna either feeds in flocks or at least in pairs and is a surface feeder and eats insects, dried chapatis, rice grains, cooked rice, cockroaches, corn and garbage waste. House crow either feeds solitary or in flocks on dried chapattis, bread, wheat grains, insects, larvae, earthworm , ticks from buffalo ears; dead rats, dogs, owlets, pigeon etc; corn, mango fruit and garbage waste. Common babbler feeds in flocks on dried chapattis, mulberry fruit and insects lying on dry leaf debris. The common myna, house crow and common babbler are omnivorous. Cattle egret feeds individually as well as in flocks of 4-5 on ticks from buffalo ears, insects, usually feeding with house crow and common myna following tractors in ploughed fields . Red wattled lapwing feeds as solitary or in flocks on insects. The cattle egret and red wattled lapwing are insectivorous. The pariah kite feeds as a solitary as well as in flocks on rat and garbage waste (which contain some fleshy material) and is carnivorous.

The excreta of different bird species was collected from the roosting, foraging and nesting sites of birds. The fresh and dry excreta of blue rock pigeon was collected from their nests as well as from the different agrifields. The dry excreta of eurasian collared dove was collected from nests taken from Ficus tree of PAU nursery. The wet excreta of rose ringed parakeet was collected from *Brassica* fields and dry from its cavity nest in Dhrek tree of agrifields. The wet and dry excreta of common myna was collected from water tunnels in the irrigated wheat fields. The wet and dry excreta of house crow was collected by spreading plastic sheets under mango trees in orchards and Pilkan tree of dairy farm, residences as well as agrifields where these birds came for feeding. The common babbler excretes on dividing branches of trees and the excretory pellets were collected from under the trees. The wet and dry excreta of cattle egret was collected by spreading plastic sheets under a Pilkan tree at dairy farm (having breeding colony of cattle egret with more than 100 nests of cattle egret) and red wattled lapwing from water tunnels in irrigated wheat fields. The dry excreta of spotted owlet was collected from Dhrek tree at Jargon Science college.

The excreta of different bird species varied from each other in colour and shape. For blue rock pigeon it was semisolid in texture, green in colour when wet and turns white on upper side and dark green to black on lower side when dry, spiral shaped and weighed 0.160± 0.02 g/ pellet. The excreta of eurasian collared dove resembles with that of blue rock pigeon in all characteristics. The excreta of rose ringed parakeet was semisolid in texture, green in colour in both wet and dry conditions, irregular in shape and weighed 0.204± 0.12 g/ pellet. In common myna and house crow it was semisolid and sometimes with partly digested grains which were also visible in dry excreta, color varies from green/ purple to brown in both wet and dry conditions. It was thin and rod-shaped and weighed 0.158± 0.07 g/ pellet in common myna and irregular shaped and weighed 0.114± 0.01 g/ pellet in house crow. The excreta of common babbler was semisolid,color being white at one and and dark grey at other end in both wet and dry conditions, rod shaped and weighed 0.193± 0.03g/ pellet. The excreta of cattle egret and red wattled lapwing was semiliquid and its color was dark brown in both when wet and dry and was irregular shaped(when collected) and weighed 0.248± 0.16 g/pellet. The excreta of red wattled lapwing was semiliquid, color was grey and white in wet condition and light grey in dry condition, rod shaped and weighed

0.294± 0.09 (g/ pellet). The excreta of spotted owlet was semisolid, creamish white (dry), round shaped and weighs 0.089 ± 0.08 g/pellet.

The excreta of six bird species (blue rock pigeon, rose ringed parakeet, common myna, house crow, cattle egret and red wattled lapwing) was tested for the presence of fungi (moulds and yeast) and bacteria (*Enterobacteriacae* family). The excreta samples of all six species of birds contained pathogenic moulds. *Aspergillus* (especially *A. fumigatus*) was detected in all six species studied. The prevalence of *A. fumigatus* was highest (86.7%) followed by *A. niger*, *A. flavus* (46.7%) , *Alternaria* sp. (33.3%) and *Geotrichum* sp. (20%) in the diverse excreta samples studied. The yeast *Cryptococcus neoformans* was isolated from fresh and dry excreta of blue rock pigeon and fresh excreta of house crow only. 73% of excreta samples showed presence of *E.coli* and 64% were positive for *Citrobacter freundii, Enterobacter cloacae* and *Kleibsella pneumoniae.* The population density of *C. freundii* was highest in rose ringed parakeet (8.2 X 10^8 cfu/ g), *E. cloacae* in red wattled lapwing (4 X 10^9 cfu/ g), *E.coli* in rose ringed parakeet (7 X 10^9 cfu/ g) and *K .pneumoniae* (3.5 X 10^9 cfu/ g) in common myna.The excreta of these bird species contains pathogens which may be harmful (mould *Aspergillus*) to these animals themselves as well as others like yeast *C. neoformans,* bacteria *C.freundii, E. cloacae, E.coli* and *K. pneumoniae* which may be a source of infection to human beings as well as may be harmful to birds themselves.

The heavy metals detected in 0.3 to 0.5 grams excreta of nine bird species(blue rock pigeon, eurasian collared dove, rose ringed parakeet, common myna, house crow, common babbler, cattle egret, red wattled lapwing and spotted owlet) in present studies included As, Cd, Pb, Fe, Cr, Cu, Ni, Zn and Mn. The concentration of all heavy metals varied from species to species. The blue rock pigeon excreted lowest amount of As (0.87±0.18 ppm), Cd (0.28±0.19 ppm), Cr (3.42± 0.12 ppm), Fe (1611±519 ppm), Ni(9.98± 1.53 ppm) and Mn (128.66± 32.87 ppm) and cattle egret excreted lowest amount of Pb(12.15±3.22 ppm), Cu (32.35±17.73 ppm) and Zn(196.94±113.53 ppm) in the faeces. However, common myna excreted highest amount of As (8.92±3.35 ppm), Cr (109.16±34.35 ppm), Fe (33788±11856 ppm), Ni (53.78±14.00 ppm), Zn (721.81±125.84 ppm) and Mn (221.78± 112.33 ppm), red wattled lapwing excreted highest amount of Cd (48.79± 19.27 ppm) and Pb (864.03± 383.83 ppm) and common babbler excreted highest amount of Cu (150.75± 110.58 ppm). This variation in levels of excreted metal concentrations may be attributed to different feeding behaviours of different species. As in the present observations the excreta was collected from the field including birds from different age groups as well as sexes which may be a factor causing a wide range in the concentration of As, Cd, Pb, Fe, Cr, Cu, Ni, Zn, Mn in these species. However, the level of heavy metals excreted in different bird species were found in toxic range as already reported for bird tissues and feathers, thus it appears that the high excretion of heavy metals like As, Cr, Fe, Ni, Zn and Mn in common myna may be a protective mechanism for better survival of a particular species as common myna which was found to be a dominating species in agrifields of PAU.

The pesticide residue levels were analyzed in 5 grams samples of excreta of different bird species (blue rock pigeon, eurasian collared dove, common myna, house crow, common babbler, cattle egret and red wattled lapwing) which included pooled samples of about 30- 50 pellets of each species. The residues of different OPs dichlorvos, acephate, phorate, monocrotophos, dimethoate diazinon, chlorpyriphos methyl, methyl parathion, fenitrothion, malathion, chlorpyriphos, chlorphenvinphos, quinalphos, profenophos, ethion, triazophos, edifenophos,anilophos, phosalone were analyzed and it was observed that all these pesticides except chlorpyriphos were non- detecbale in excreta of all bird species. The only pesticide detected was chlorpyriphos which was found to be in concentration of 0.046 ppm in house crow and 0.33 ppm in cattle egret. The OCs residues such as α-HCH, γ –HCH, β-HCH, heptachor, δ-HCH, aldrin, dicofol, op DDE, α- endosulphan, DDE, DDD, DDT, β-endosulphan, pp DDD, pp DDT, endosulphan sulphate and synthetic pyetheroids residues like bifenthrin, fenpropathrin, γ- cyhalothyrin, β- cyfluthrin, cypermethrin, fluvalinate, fenvalrate and deltamethrin were not detected in excreta of any bird species.

Chlorpyriphos is an OP which severely affects the birds. Its detection in excreta of crow and cattle egret in present studies may perhaps be because of it being a most commonly consumed pesticide in India as well as in Punjab. Its detection in excreta is present studies may be correlated with its common use in insecticidal formulations for the control of ticks in cattle and buffaloes at dairy farm of GADVASU from where the excreta of cattle egret and to some extent crow was collected. Thus it throws light on a direct correlation between the use of chlorpyriphos and its entry into these birds through oral as well as dermal routes by consumption of ticks while sitting on cows and buffaloes.

It can thus be concluded that the bird species i. e. blue rock pigeon, eurasian collared dove, rose ringed parakeet, common myna, house crow, common babbler, cattle egret, red wattled lapwing and pariah kite are grainivorous, frugivorous, omnivorous, insectivorous or carnivorous in nature and pass their excreta at the roosting, foraging and breeding/ nesting sites. Microbial analysis revealed the presence of pathogenic mould *Aspergillus,* yeast *Cryptococcus neoformans,* bacteria *Citrobacter freundii, Enterobacter claocae, E.coli* and *Kleibsella pneumoniae* which may/may not cause disease in these birds but their presence in excreta shows that they can transfer these pathogens to human beings and other animals.

Since the beginning of the use of chemical insecticides in fields of agriculture and human health, a great impact has been thrown by acute and chronic effects of POPs like OCs because of their high bioaccumulation and later by use of less persistent but more potent OPs on bird diversity and population world over. As the pimary pressure of environmental impact on declining bird population is chemical pollution including pesticides and toxic metals at sublethal levels (as also reported in the present studies) insufficient to produce outright mortality or other acute toxic effects which may neverthless lead to profound consequences on birds such as increased reproductive dysfunctions like egg shell thinning , endocrine disruption and increased susceptibility to diseases or other stresses. Because of the multiple routes of exposure of birds to chemical pollutants as well as the limited capability of birds to perform certain detoxifying transformations, the excreta may not be a very good monitoring tool for correlating the pesticide exposure and its effects on bird population decline.

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