Effect of pollutants and heavy metals on pond heron, *Ardeola grayii*

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This thesis is dedicated to my family and teachers

CANDIDATES'S DECLARATION

I hereby declare that the thesis entitled **"Effect of pollutants and heavy metals on pond heron, *Ardeola grayii*"** submitted by me for the degree of **Doctor of Philosophy** in **Applied Animal Sciences** is the result of my original work carried under the supervision of **Dr. V. Elangovan**, Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow and it has not been submitted for the award of any degree, diploma, associateship of any university or institution. I also declare that this thesis is essentially free from all kinds of plagiarism.

Akanksha Dwivedi

Signature of candidate

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Date: 02/11/2021

Place: Lucknow

CERTIFICATE

This is to certify that the thesis entitled **"Effect of pollutants and heavy metals on pond heron, *Ardeola grayii*"** submitted by **Ms. Akanksha Dwivedi** is an original work and has not been submitted for the award of any degree, diploma, associateship of any university or institution.

The thesis submitted to Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, satisfied the all requirements as stipulated in the *Doctor of Philosophy (Ph.D.) regulations - 1999 as amended in 2013* and it is fit for submission and evaluation for the award of the degree Doctor of Philosophy of the University.

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LIST OF ABBREVIATIONS

°	:	Degree
ha	:	Hactare
°C	:	Degree Celsius
Km	:	Kilometre
Cm	:	Centimeter
Min	:	Minute
%	:	Percentage
Hrs.	:	Hours
Mg/L	:	Milligram per liter
Nm	:	Nanometer
Ml	:	Milliliter
µg/L	:	Microgram per liter
ppm	:	Parts per million
gm	:	Gram
µg/g	:	Microgram per gram
SD	:	Standard deviation
H	:	Kruskal - Wallis H test
F	:	One-way ANOVA
ICAP-AES	:	Inductively coupled plasma atomic emission spectroscopy

General Introduction

India is known for varied geomorphic and consequently rich biodiversity continent. In animal species birds play a major role in ecological ecosystem, they are easy to observe and monitor. There are about 9000 species of birds distributed throughout world. According to the International Ornithologists Union, the global diversity has approached 10,425 bird species (IUCN Red List of Threatened Species, 2015-4) and among them, 1340 (13%) bird species were observed from India. As India is one of the 12 mega biodiversity countries, supports a considerable amount of avifaunal population. Birds feed on various harmful insects and agricultural pests, thus they support farming system and human beings, and also act as pollinator, scavenger, and as bio-indicator, because it is sensitive to environmental changes (Manral and Khudsar, 2013). Wading birds are wetlands dependent, these are group of small medium sized birds, having a wide variety of bill structures, possess long legs and toes enabling them to live and feed in shallow water. It is belonging to following families, viz. Ardeidae, Charadriidae, Recurvirostridae, Gruidae, Rallidae, Ciconidae, Jacanidae, Threskiornithidae and Burhinidae (Kushlan, 1976). Morally we have no right as human being to make perilous environment for the other species of natural world that have the same right to exist with nature as much as human society.

There are sixty-five species of herons recognized throughout the world (Kushlan, 2007). It was assessed and found that 8 species are currently under threat and 2 species are near threatened (Kushlan,2016). Four species of the herons are extinct in historic times, due to habitats degradation, it is the main cause of threat to heron. Previous studies report herons are an adaptable group of birds

(Kushlan, 2007) and most of them have been able to co-exist with human in their natural ranges. Birds play important role in indicators of the ecological conditions and productivity of an ecosystem (Desai and Shanbhag, 2007). Avifauna is one of the important components of food chain in environment (Ali, 2002).

They serve an important ecosystem function of accelerating the nutrient cycling in the feeding grounds and balancing the fish populations, the availability of food is of primary importance to the birds during migration when they store energy for winter and successful completion of migration (Tak *et al*., 2003).

Indian pond heron is distributed throughout the Indian union, Bangladesh, Pakistan, Sri Lanka and Myanmar, and upto 1000m elevation (Thakur and Mehata, 2014). *Ardeola grayii* is a long legged wetland bird species belonging to the family Ardeidae under the order ciconiiformes. Indian pond heron is chiefly earthy brown with the glistening white wings, tail and rump flashing into prominence immediately it flies, juvenile have light green colour leg, in breeding season acquire maroon hair like plumes on back, and sometime red leg appear for short period, sexes alike. They are the inhabitant of ponds, pools, rivers, stream, tidal flats, flooded grassland, paddy fields, canals, and ditches. Unlike the majority of herons that feeds typically in flocks (Bildstein, 1984). *A.grayii* generally feeds solitary but sometime seen with small groups. Behaviour is commonly considered to be one mechanism of resource partitioning (Recher and Recher, 1969), presumably based on the idea that herons capture prey in different ways and use different part of feeding habitats or different depth of wetlands, some feed near edge of wetland, some in depth. The foraging activity of birds is necessary for their survival and reproduction, making it an essential aspect of the life of birds (Papakostas *et al*., 2005). Foraging is a multidimensional behaviour; herons use a varied foraging repertoire to catch prey (Rodgers, 1983). Kushlan (1978) has reported 38 feeding behaviour relevant to Ardeidae species. Most frequent used behavior of Indian pond heron is sit and wait , where a bird would motionless for long periods before it struck at prey items or else moved to another location. Regardless of whether they struck at prey or not, some pond heron remained at a single location for entire day, whereas some of them relocated one or more times. Some factors which affects the overall activities of *A. grayii*, such as structure of habitats, vegetation, water depth, availability of prey, types, size, weight of prey and types of techniques for capturing prey etc. A habitat includes all four necessities for a bird's survival these are food, water, shelter and nesting areas though these features can vary greatly between different type's habitats. In addition, to having the appropriate features to support bird survival, a habitat also includes all the associated landforms (mountain ranges, coasts, plateaus, valleys, etc.), seasonal climate patterns, predators and other wildlife.

Prey species and its availability determine whether foraging birds should adopt a specialist or a generalist strategy (Kushlan, 1978). The mode of feeding depends on type of prey species, i.e. whether they are bottom dweller or surface foragers. Environmental variables clearly influence the foraging behaviour of wading birds (Rodgers, 1983; Bates and Ballard, 2014). Bird species that face seasonal fluctuation in availability of food have two alternatives; they may shift to feeding on other food resources, or may move to other area where original food resource such as fishes, insects, amphibians, annelids are available (Karr, 1976). Seasonal variation in resource availability plays dominating role in evolution of species and communities (Beals, 1970; Fogden, 1972; Leck, 1972). Water level is another factor which influences the selection of habitat by pond heron, because it influenced prey availability and might account in part for differences in the sizes of foraging groups and feeding success.

The quality of living for an aquatic life and organism which depends on it, influenced by the water quality (Kulshrestha and Sharma 2006; Mulani *et. al*., 2009). Water quality assessment generally involves analysis of physical, chemical, and biological parameters and reflects on abiotic and biotic status of the ecosystem (Kumar and Singh, 2002). Water forms the life of all the living organisms on Earth planet. Water quality obviously plays a vital role in this relationship, as it is key to the maintenance of a well-balanced environment. A balanced ecosystem is one in which living things and the environments interact beneficially with one another. Alteration in the wetlands may affect the vital activities of wading birds (Kumar and Gupta, 2013). Wetlands and wading birds are inseparable elements (Grimmet and Inskipp, 2007).

Industrial wastewater and domestic sewage are directly discharged into wetland water without standardized treatment. As a result, Birds ingulf polluted or contaminated food that is detrimental to their health, further leading to a large number of deaths due to some toxic substances present in feeding area. There are many reports published by Burger and Gochfeld (1991), Beale and Monaghan (2005), they explained how non-environmental friendly products used by humans affect wetland birds.

Unpolluted or a healthy wetland maintained higher bird density and diversity than polluted ones. The developmental processes and anthropogenic activities affect the habitat structure of an area which in turn affects natural property of wetlands and biodiversity (IPCC, 2014). Pollution of the environment is one of the terrible ecological disaster to which they are subjected nowadays. Nearly all of the activities of human society have produced unfavorable effects on all living forms in the biosphere. The cause of water pollutants are domestic sewage, detergents, pesticides, chemicals, dead materials and industrial effluents through a variety of processes (Sampath and Sharam, 2003). Birds are at or near the top of food chain and consequently suffer from accumulating environmental pollutants. Because heavy metals in the feathers and eggshells of birds reflect the background abundance of these pollutants, birds can be a useful indicator of heavy metal pollution in the environment (Burger, 1996). The presence of heavy metals in air, soil, and water has a serious impact on living organisms and is extremely dangerous because of their bioaccumulation and amplification in the food chain. For example, in the transfer of Phytoplankton→Copepods→Fish, a typical marine food chain, mercury concentrations increase with trophic levels (Berglund *et al*., 2011). Heavy metals entering the water body firstly absorbed by sediments and then move to water, sediment and biota, through biological and chemical process. Heavy metals do not degrade in water as a result it is accumulated in aquatic organisms. Birds are exposed to heavy metals through air, water and their food. Once a metal has entered the body it can be stored or accumulated, or be excreted. The Ardeids are likely to have accumulated lead as well as chromium from their foraging grounds in the wetlands near their nesting sites. Non-essential metals such as Cd, Cr, Pb, and Hg have an adverse effect on the body mass and health of birds thus reducing growth or body weight showing depressing impact on their survival and reproductive success (Dauwe *et al*., 2004). Avian species are useful bio-indicators of metal contaminants due to their sensitivity to anthropogenic contamination (Martinez, 2000). Feather is connected with the bloodstream through small blood vessels and metals can bind to the protein molecules in the feather with its growth. After the feather is fully formed, the blood vessels atrophy and the feather becomes physiologically separated from the bird (Dauwe *et al*., 2004), resulting in a fairly constant metal concentration in feathers (Martinez, 2000). Therefore, feathers are ideal for studying heavy metal pollution in ecosystems. Female birds can eliminate metals by sequestering them in their eggs, especially eggshells (Dauwe *et al*., 1999). Because eggshells can adequately reflect the environmental levels of metals, they have been used to monitor the exposure of bird populations (Dauwe *et al*., 1999; Mora, 2003). Furthermore, feces are important way for birds to excrete metals from their bodies and higher concentrations of metals are usually found in fecal samples (Berglund *et al*., 2011). Therefore, feces are also useful bio-indicators for bio-monitoring of heavy metal pollution in birds. It can get rid of heavy metals from their body through the feces or by depositing them in the feathers. Martinez, (2000) and Metcheva *et al*., (2006), observed concentration of arsenic and mercury in feathers, eggshells and feces in seabirds. The main form of Hg in feathers is Methyl-Hg, which is beneficial to its accumulation in the keratin structure of the feathers. Cd, Ni and Pb are store in eggshells. Dauwe *et al*., (1999) indicated that the non-essential elements such as Pb were more likely to enrich in eggshells rather than egg contents. Chromium, Cu and Zn were mostly excreted through feces.

The main sources of metal pollutants are industrial mining, domestic and agricultural wastewaters, which are discharged into rivers and oceans, and the metal pollutants can be strongly accumulated and biomagnified along water, sediment, and aquatic food chains (Yi *et al*., 2011). Due to the position at the top of food chains and the spatial integration of contaminant levels, birds are usually used as bio-indicators of environmental pollution (Denneman and Douben 1993; Dauwe *et al*., 2004). The contaminant levels in birds can reflect the anthropogenic activities in some extent. The heavy metals enter the feathers and will not easily be degraded or excreted. The metals concentrations in feathers indicated the local level of heavy metal emissions.

Therefore, the study was carried out on following objectives;

1. The food composition and feeding behaviour of *Ardeola grayii*.
2. To study the pollutants of ponds and wetlands and their effects on behaviour of *Ardeola grayii*.
3. To study the accumulation of heavy metals on diets, feathers, and feces of *Ardeola grayii.*

Review of Literature

The behavioural ecology of birds deals with all adaptive circumstances such as roosting, foraging, reproduction, parenting behaviour. The foraging behaviour is most important behaviour for their survival and reproduction, thus it plays a vital role in fitness of birds **(Nefla and Nauira, 2016).**

Herons mostly live their life near water bodies such as lake, pond, river and canal. Foraging activity is an essential aspect of life of birds, being necessary for their survival and reproduction **(Kushlan, 1979).** Feeding habitat and feeding techniques are important factors engaged in resource separation and can be used to understand feeding guilds of the birds.

Indian Pond heron prefers to feed near lakes, ponds, ditches, marshes, river, canal, and garbages. These habitats provide birds shelter, food and other necessity for their survival. Pond heron captures more small prey in marshes than lake, and after summer outbreak improved marshy land increase food availability for them. The food composition of pond heron depends on availability of prey and type of habitats. it generally feed on fishes, frogs, insects, annelids, crustaceans and sometime seen near garbage to feed on worm **(Anthal and Sahi, 2017).**

Foraging behaviour and success of herons affected by habitats, where they feed. Pond herons use various techniques to capture prey, and they also depend on prey type, structure of habitat such as depth of water, presence of vegetation. Water level and availability of vegetation in feeding habitats contribute to the abundance of prey **(Jayson and Shivaperuman, 2011).**

Feeding strategies such as, walking slowly and quickly, disturb and chase, probing, scanning, stand and wait, bait fishing, crouched position, bill dipping, are used by pond herons depend on size and nature of prey, habitats, and seasons **(Bates and Ballard, 2014).**

Thirty-eight feeding behaviour of herons were reported by **Kushlan (1978)**, out of which the most frequent used behaviour is stand and wait. Herons most often hunt from an upright position but occasionally it uses crouch and wait and crouch and walk slowly techniques for hunting. In crouched position birds keep their body in lower position and hold it in horizontal to the substrate state.

Herons shifts their habitat in search of food. In herons some are solitary feeder and some are flock feeder**,** flock feeder herons has significantly higher rate of feeding success than solitary feeder. **(Chaskda *et al*., 2018).**

Time budget studies of herons foraging has been reported by many authors. Herons are mostly diurnal feeder, but some of them feed during night. Time of feeding is also divided into three times zones, i.e. in morning, afternoon and evening. Time of feeding varies according to seasons, such as in summer pond heron comes in fields in early morning and feed till evening, but in winter they come late in the morning and feeding stop before sunset **(Porte *et al*., 2018).**

The time spent in foraging does not differ between foraging techniques infact tactile and visual feeding species usually foraged in intra-species or interspecies flocks **(Asitava and Subhra, 2019)**. Foraging in groups is beneficial because it reduces the risk of predation and thus reduces the cost of vigilance. Species with different foraging techniques generally acquire food resources form different habitat and may get able to avoid interspecies competition.

Ardiedae generally feed during day time in open aquatic habitats and capture fish and other small insects. Some common features shared by all Ardiedae are morphology and share roost colonies, and feeding areas. Their preference for selection of habitat is variable e.g. Egrets adapts terrestrial habitats, swampy, marshy and rice field area but Indian pond heron and Grey heron adapt open large aquatic habitats **(Meganathan and Jeevanandham, 2018).**

Some researcher has found out that day feeder uses visual feeding techniques for capturing fish. Foraging attempts increase as availability of prey widespread because of increases in the water level. Water level conditions reflect major changes in the abundance and availability of food that are present in temporary, seasonal and semi-permanent wetlands. As summer arrives herons foraging attempts decreases due to the decrease in number of fishes **(Novcic and Beauchamp, 2018).**

Chapter II

Aquatic environment is valuable natural treasure. Aquatic ecosystem is the most diverse ecosystem in the world. The healthy aquatic ecosystem depends on the physico-chemical and biological characteristics. The quality of water in any ecosystem provides significant information about the available resources for supporting life in the ecosystem **(Gursimran *et al*., 2018)**. The physico-chemical environment of water functions in many ways and employs the influences upon biotic components, thus gives a picture of the environmental suitability of water to maintain its life.

Birds are good model for examining pollutants levels over wide geographical areas because they often are on the top in food chain and their life span is longer as compare to other organism allowing bioaccumulation and detection detrimental levels. Many aquatic birds are regarded as bio-indicators of heavy metal contamination in aquatic environments.

The distribution of heavy metals in water, sediments, and aquatic organisms play a vital role in detecting the sources of metal pollution in aquatic ecosystem. It is an established fact that maintenance of healthy aquatic ecosystem depends on physico-chemical parameters of water and biological diversity. pH value of water also affects the metal toxicity in several ways **(Malik and Zeb, 2009)**.

The sewage and industrial effluents causes increase in hardness of water and nitrate concentration, which result in decrease of pH values and increase of hardness of water.

Metals are found naturally in aquatic ecosystem by a wide range of natural and anthropogenic source and with anthropogenic activity being either domestic or industrial. The availability and toxicity of these metal are immensely influenced by the physico-chemical factors of the specific environment.

Water birds who spend most of their life cycle near the wetlands get directly or indirectly affected by water pollutants. In a study it was found that polluted ponds are less occupied by wetland birds as compared to the unpolluted ponds **(Pandotra and Sahi, 2014).**

Behaviour is suggested to be more useful indicator or biomarker than standard assay in laboratory conditions because the harmful effects of pollutants sometimes become only noticeable in natural ecological conditions, such as social stress or infections. The behaviour is the outcome of a developmental and physiological processes, so it should provide a more inclusive measure than one or a few chemical, physical or morphological parameters **(Gorrisen *et al*., 2005).** Metal contamination affects singing behaviour of some birds.

In some herons and egrets behavioural changes can be easily seen due to degradation or less availability of waterbodies for their survival. Variation in flight initiation and distance are an important measure for analysing a bird tolerance to urbanization **(Roshnath, 2015).** Some birds adopt urban condition and tolerate human presence while some birds completely isolate themselves from such conditions and maintain a distance from all such things.

Metal concentration is increasing in waterbodies due to discharge of untreated effluents in rivers, which are mostly consist of sewage wastage, domestic wastage, hospital wastages, industrial effluents and many other anthropogenic wastages. These contaminants of heavy metals may sometimes cause detrimental effects on ecology and diversity of aquatic fauna **(Aggarwal, 2000).**

Metal pollution in wetlands not only affect the human health, but may also affect aquatic life and associated wildlife **(Ayas and Kolankaya, 1996).**

Chapter III

The distribution of heavy metals in water, sediments, and other living organisms such as aquatic organisms and water birds play a key role in detecting the sources of heavy metal pollution in aquatic ecosystem. Metals naturally originate through natural ecosystems, perhaps the human induced causes of metals such as resource extraction, agricultural pollutants, residential pollutants and environmental accumulation significantly increases the concentration of metal especially in the aquatic ecosystem.

Traced elements are categorized in two groups essential elements and non-essential elements out of which essential elements are required for the vital functioning of the organism where as non-essential elements are not needed for any physiological function but are highly toxic if once accumulated inside the organisms **(Berglund *et al*., 2011).**

Cadmium is one of the most abundant non-essential metals present in the environment because of its use in industrial activities. If Cadmium is absorbed in the organism through the digestive and pulmonary system, it 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 **(Kim and Oh, 2012).**

For avian species Lead is more dangerous than other metals. It enters through grit, soil intake from preening or ingestion of contaminated food. Sources of Lead in the environment include fuel additives, lead pigments in paints, batteries, pipes, and glazed ceramic food containers. In birds it causes delayed parental and sibling's recognition, locomotion, impaired thermoregulation, lowered nestling survival and abnormal feeding behaviour **(Zala and Penn, 2004).**

Chromium (Cr), Manganese (Mn), and Zinc (Zn) are essential elements. Cr naturally presents in rocks, plants and soil and sometimes generally produced by industrial processes. Cr produced adverse effect on embryonic development, hatching and viability of birds **(Manjula *et al*., 2015).**

Mn supports the immune system, regulates the blood sugar levels and is involved in the production of energy and cell production. It has adverse impact on avian embryo, limb growth, neck defects **(Manjula *et al*., 2015).**

Zn is an important component of the enzymes responsible for metabolism of proteins and carbohydrates. The sources of Zn are cloth clips, staples, zippers, keys, nails, antirust paints, shampoos, and skin preparations. It causes dysfunction of liver, pancreas, kidney and even result in death **(Kim and Oh, 2012).**

When birds are used as an indicator species for metal toxicity, non-invasive method is used to detect it. This method does not disturb or kill any birds. In this method, the feathers, feces, food, and eggshell of birds are used for analysis purposes. The Ardeidae are family of water birds residing in wetlands and inland water areas and feeding on fish and invertebrates. Due to their position at the top of the food chain, they are particularly vulnerable to heavy metal toxicity **(Lantz *et al*., 2010).**

Feathers of birds are useful tool for monitoring metal contamination and their collection is collectively non-invasive and replicated on the same individual overtime **(Markowski *et al*., 2013).** Feather reflects the amount of metals present in the blood at the time of feather growth, either from current dietary sources or from mobilization of metals from internal organs. Feathers are connected with the blood stream through the small blood vessels and the metals can bind the protein molecules in the feather during its growth. However, the metal concentrations in feathers not only reflect their dietary intake but also the external contamination.

Birds can rid the body of heavy metals through the feces **(Dauwe *et al*., 2000)** or by depositing them in the uropygial gland, salt gland and feathers. Concentration of metal present in birds depend on several factors such as, age, sex, size of birds and availability of food materials in different habitat sites from where the sample were collected.

The feces and food of birds can be a useful bio-monitor for heavy metal pollution. Analysis of metal through feces does involve killing and disturbing the animals. The metal content in birds' feces reflects the metal concentration in food and the ambient environment. Insectivorous and piscivorous birds living in polluted habitat are not only exposed to pollutants but they may also be affected by changes in their diet. **(Ayas and Kolankaya, 1996).**

Chapter-I

Food composition and feeding behaviour of pond heron, *Ardeola grayii*

CHAPTER-I *Food composition and feeding behaviour of pond heron, Ardeola grayii*

INTRODUCTION

Birds are one of the healthiest indicators of the ecological conditions, productivity, trophic structure, and contamination of wetlands. Birds are important to continue ecologic circle, specially food chain. For survival of any living organism, it requires considerable amount of energy. Feeding is a crucial activity of the bird's life which is vital for its survival however the demands of food acquisition impose significant challenges to both physiology and behaviour of birds. The bird species, directly and indirectly, depends on their habitats for several purposes like feeding, breeding, nesting. Wading birds are most commonly associated with wetlands, streams, and other aquatic habitats such as ponds, lake etc. The members of family Ardeidae are medium to large size wading birds, consist of 9 species viz. *Ardeola grayii* (Indian Pond Heron), *Nycticorax nycticorax* (Black- crowned Night Heron), *Ardea cinerea (*Grey Heron), *Ardea purpurea* (Purple Heron), *Egretta garzetta* (Little Egret), *Bubulcus ibis* (Cattle Egret), *Mesophoyx intermedia* (Median egret), *Casmerodius albus* (Large egret) and *Ixobrychus cinnamomeus* (Chestnut Bittern). These wading birds capture prey by employing stand and wait strategy (Kushlan, 1976; Anthal and Sahi, 2017), or by walking slowly towards the prey and waiting for surprise attack (Kushlan, 2005).

The bird selected for this study is Indian Pond Heron (*Ardeola grayii),* which is member of the family Ardeidae (Order Pelecaniiformes). It usually feds on worms, grubs, frog, fish, crabs and insects. It has adapted itself well to live alongside humans and can be seen both in the rural and near the vicinity of urban habitations.

Ardeola grayii (Indian Pond heron) mostly depends on fishes and frogs (Sodhi, 1992) but during breeding season, it preferred to feed on fishes (Roshnath, 2015). *A. grayii* is a long-legged wetland bird species and commonly known as Paddy bird. *Ardeola grayii* is solitary feeder and occasionally with small groups in all types of wetlands (Roshnath, 2015; Santharam, 2003) by its long bill. Their camouflage in the natural habitat is so excellent that they can be approached closely before they take to flight, which is a kind of behaviour that has resulted in folk names and beliefs that the birds are short sighted or half blind and hence called "Andha Bagula". It is used many techniques to capture prey such as; walk slowly, surprise attack (Kushlan, 2005), fish baiting using bread crumps (Reglade *et al*., 2015), scavenging behaviour (Roshnath, 2015), stand and wait (Anthal and Sahi, 2017), probing (Porte *et al*., 2018), floating behaviour (Greeshma and Jayson, 2018). Feeding behaviour and their efficiencies in herons are affected by several factors including prey density and prey availability (Richardson *et al*., 2001), seasons (Lekuona, 2004), habitat characteristics (Matsunaga, 2000) and height of vegetation (Lantz *et al*., 2010).

Seasonal variation in food abundance often influences the use of different habitats while seasonal rainfall changes the availability of food, geomorphology of habitats and seasonal crops (Beals, 1970; Fogden, 1972; Karr, 1976). Seasonal variation in resource availability plays a dominating role in the evolution of species and communities (Beals, 1970; Fogden, 1972; Leck, 1972). For most of wading birds, critical seasonality is a wet and dry cycle of weather (Kushlan, 1978). Availability of aquatic prey may vary with seasons but this also depends on the fluctuation in water level condition. The availability of prey directly or indirectly affected by wet and dry conditions of seasons (Kushlan, 1978; Kushlan, 1986) that can be a major limiting factor of avian populations (Skutch, 1949; Lack, 1954). Many studies have shown that prey availability within a habitat is important in determining wading birds for selection of a foraging sites (Baidu *et al*., 1998; Laubhan and Gammonley, 2000). Besides the season and availability of prey, water depth can also affect both accessibility of foraging habitat and the vulnerability of prey to wading birds.

As per the Indian Wildlife (Protection) Act, 1972, Herons are common species placed in the Schedule IV. Under the present scenario of research in wildlife studies, most of the studies are oriented towards endangered and threatened species; but the common birds are mostly forgotten. But in the history of conservation, this ideology has been documented to be a mistake, as reviewed by the fate of Imperial Pigeon in Europe (Maheswaran and Rahmani, 2002) and the Vultures and House Sparrow in India and elsewhere. Furthermore, information available on local birds when they are fairly common can provide a baseline model for their future conservation if and when need arises in case they ever confront threats to their survival. There are no ample studies available on the foraging ecology of Indian pond heron, only a few studies documented (Andrew and Mathew, 1997; Sawara *et al*., 1994; Santharam, 2003; Begum, 2003; Seedikkoya *et al*., 2012). In India, most of the work was done on their breeding biology, but very few studies carried out on foraging behaviour including feeding success, food composition and feeding behaviour. Therefore, this study was aimed to access the food composition, prey abundance, foraging success, and feeding behaviour of *Ardeola grayii* in different habitats and seasons.

MATERIALS AND METHOD:

Study area

The present study throw light on feeding behaviour and food composition of *Ardeola grayii* was carried out in different area of Lucknow. The location of Lucknow is in the state of Uttar Pradesh in India situated on the northern gangetic plains of India, Lucknow is the capital city of Uttar Pradesh. The geographical location of Lucknow is between 26.50 North and 80.50 East. Lucknow is located at an elevation of 123 meters above sea level. The city was known by the name of Awadh during ancient times. Lucknow is situated in the heart of the great gangetic plain, surrounded by its rural towns and villages like the orchard town of Malihabad, historic Kakori, Mohanlalganj, Gosainganj, Chinhhat, Itaunja. On its eastern side Barabanki, on the western side Unnao, on the southern side Raebareli, and on the northern side Sitapur and Hardoi districts. The Gomati river the chief geographical feature meanders through the city, divinding into the trans Gomati and cis-Gomati regions. Feeding sites of *Ardeola grayii* and its GPS location and area (hectare) was given in Table1.1.

Table 1.1. GPS location and area of feeding sites of *Ardeola grayii*

Study site	Location	Habitat type	Area
Kalli paschim	2644’16” N, 8057’18”E	Lake	3 ha
Babu khera	2644’25” N, 8057’31”E	Grassland	8ha
Vrindavan	2644’16” N, 8057’18”E	Marshy area	10ha
Jagat khera	2644’30” N, 8058’1”E	Grassland	6ha
Mawaiya	2645’31’’N, 8056’32’’E	Lake	10ha
Behasa	2645’33’’N, 8051’18’’E	Pond	7ha
Nawabganj	2637’09’’N, 8039’11’’E	Lake	224.60 ha

Barabanki	2655’36’’N, 819’41’’E	Agriculture field	17ha
Barabanki	2654’33’’N, 819’52’’E	Agriculture field	20ha
Unnao	2632’49’’N, 8028’29’’E	Agriculture field	3ha
Unnao	2633’21’’N, 8030’38’’E	Pond	0.5 ha
Amausi	2645’34’’N, 8051’21’’E	Pond	2 ha
Chandrika devi temple	271’20”N, 8050’7’’E	Pond	8ha

Climate

Lucknow has warm humid subtropical climate with cool, dry winters from December to February and dry hot summer from April to June. The rainy season is from mid- June to mid-September, when Lucknow get an average rainfall of 1010 mm (40) mostly from the south-west monsoon winds. There are three marked seasons in a year: with heat wave. In this study maximum temperature 40-45℃ to minimum 32℃.

Summer seasons- It starts from March to June, **Monsoon seasons**- It starts in mid of June, but generally good amount of rainy starts in July, so from July to October monsoon season begin. **Winter seasons-** It starts from November to February. In this study maximum temperature ranges from 28-30℃ and minimum 6-8℃. The data for temperature and humidity was collected in different habitats of *Ardeola grayii* through thermo-hygrometer (Table. 1.2).

Table 1.2. Average temperature during summer, monsoon and winter seasons at lake, marshy area, pond, grassland, and agriculture field. All values are given in mean ± SD.

Habitat	Summer	Monsoon	Winter
Lake	33.75 ± 2.73	35 ± 1.8	20.91 ± 2.77
Pond	38.75 ± 3.38	57.25 ± 2.25	23.66 ± 3.05
Marshy area	38 ± 3.76	34.66 ± 1.61	28.83 ± 1.99
Grassland	40.16 ± 1.52	33.91 ± 2.02	24.5 ± 2.43
Agriculture land	39.75 ± 3.38	33.16 ± 2.28	23.91 ± 2.6

Table 1.3. Average humidity during summer, monsoon and winter seasons at lake, marshy area, pond, grassland, and agriculture field. All values are given in mean ± SD

Habitat	Summer	Monsoon	Winter
Lake	39.66 ± 9.44	64.41 ± 8.51	71.8 ± 17.21
Pond	55.58 ± 15.81	57.25 ± 2.25	82.83 ± 16.7
Marshy area	64.83 ± 10.43	70 ± 15.59	56.25 ± 16.42
Grassland	31.25 ± 9.99	76.91 ± 14.16	65.83 ± 11.65
Agriculture land	45.83 ± 15.46	87.41 ± 6.1	54.41 ± 15.35

The observation period was divided into three time zones; Morning (6:00h to 10:00h), Afternoon (11:00h to 2:00h), and Evening (3:00h to 5:00h), and every activity or behaviour recorded for five days in a week at 15-minute interval, data were recorded in excel sheet. In this study, five different types of habitats were selected randomly including natural and man-made, these were described according to Ramsar convention, (2012) Lake, Pond, Marshy area, Agriculture field and Grassland, all these sites are situated in different area of Lucknow from east to west and north to south, and distance from main city was up to 50 km. Longitude, latitude and distance from main city for all study sites given in Table 1.1.

Lake: Lake is deep water body surrounded by woody and non-woody tree habitats of many flora and fauna. In this study site, the lake is surrounded by *Azadirachta indica, Eucalyptus globules*, *Mangifera indica, Vachellia nilotica, Ipomoea carnea, Eichhornia crassipes, Pithecellobium dulce* etc.

Marshy areas: Marshy area was flooded during rainy and in summer dried out. In marshy area mostly grasses, and low growing shrubs flourish.

Pond: Pond is shallow, permanent or semi-permanent water with little flow; in this study site, the pond is near to temple with many surrounding trees such as *Ficus benghalensis, F. virens, F. racemosa, Mangifera indica, Azadirachta indica* etc.

Agriculture field: Agriculture field is artificial wetland in Ramsar classification it comes under irrigated land, according to Ramsar classification it is flooded naturally or artificially, in this study main crop grown in field was wheat and paddy, and these site was used by *Ardeola grayii* during all seasons.

Grassland: Grassland is barren area without any building or large tree, very few grass and shrubs present there, mostly *Ardeola grayii* used these habitats during winter.

The water depth and height of vegetation present in lake and pond was measured by wooden scale, when *A. grayii* left feeding habitat. Unit of measurement was centimeter.

Methodology

Prey abundance and food composition

For the seasonal variation in prey availability, aerial prey and benthic prey were collected from foraging sites on all five study sites once in a fortnight. The prey items were identified and recorded followed by literature. Prey items were sampled by 5 sweeps (Tojo, 1996; Fasola, 1986) of a long-handled net from the water edge randomly between 0 and 5 meters, distance from water edge was measured by meter stick. Fishes were collected with the help of fisherman. Aquatic insects and fishes were identified following (Saksena and Kaushik, 1994; Jayaram, 1999) respectively. The size (length and weight) of prey items was measured with a scale, and released at the site of capture. Food composition of *A.grayii* was done by direct observation with the help of binocular (Celestron 10×50X and digital camera (Sony and Nikon Coolpix) from 100- 200 meter distance.

Feeding frequency (Foraging attempt /success)

Feeding activities of *A. grayii* was observed using binocular (Celestron Upclose G2, 6.8°/354FT/118m (10×50X) and digital camera (Sony and Nikon Coolpix). Observation was done from 100-200m distance without disturbing bird. Observations were stopped if focal individual go away or flew away from site. Feeding frequency of *A. grayii* recorded for three hours per day for five days every month without disturbing their activities. Feeding frequency was counted with a stopwatch and whenever possible through visually for each 15 min feeding bout (15-minute observation whether a prey species was captured by pond heron or not). A 15 min foraging attempt was considered successful if *A. grayii* caught any prey items, and was considered attempt without success if it failed to do so. **For calculating success ratio, foraging success was divided by foraging attempts (success/attempts) for every 15 minute of observation. Success ratio = No. of foraging success/ No. of foraging attempt (for every 15-minute success and attempt was calculated)**

Feeding behaviour

The feeding behaviour of *A. grayii* was observed for five days in every week two hour continuously with 15 minute break, and observation were conducted during four-interval period i.e. 0700-0900hours, 0900-1100hours, 1100-1300hours and 1300-1500hours. The behviour was recorded in camera, for analysis, video was played back in slow motion. For observation, focal animal sampling method was used described by Altman (1974).

The terms used to describe various feeding behavior of A.grayii was followed by; Kushlan, 1978; Tojo, 1996; Richardson et al., 2001; Porte et al., 2018; and Greeshma and Jayson, 2018). Feeding behaviour such as a stand and wait, walk quickly, diving, bill dipping, crouched position, neck movement and Hopping were recorded.

Statistical analysis

All statistical analysis was performed using SPSS (version 21.0). Normality and homogeneity were evaluated for the distribution of data sets. $P < 0.05$ was considered as statistically significant. Prey abundance of lakes, ponds and marshy area was found normally distributed ($p < 0.05$) except amphibians of lakes and annelids of ponds ($p < 0.05$). Data of foraging success of Indian pond heron in all three seasons in lakes and ponds for amphibians, annelids and molluscs for the lake, ponds and marshy area were found not normal ($p > 0.05$) while the rest of them were normally distributed

($p < 0.05$). Crouch position and Neck movements were found not normally distributed ($p > 0.05$) while the rest of feeding behaviour were found normally distributed ($p < 0.05$). A non- parametric test (Kruskal - Wallis) was used when data did not fit a normal distribution, otherwise parametric test that is One-way ANOVA used to evaluate the differences in prey abundance, foraging success and feeding behaviour of *A. grayii* in five different habitats and seasons. We used curve fitted model to show different pattern of foraging attempt and success in different seasons and habitats. Before using curve fitting model we plot scatter plot, for checking data are linear or non-linear, if data are non-linear, then we use curve fitting model. In curve fitted model we have used all model such as linear, logarithmic, inverse, quadratic, power, exponential, and logistic, the model that has the lowest value of residual sum of squares we use it. Curve fitting refers to finding an appropriate mathematical model that expresses the relationship between a dependent variable *Y* (success) and a single independent variable X *(*attempt) and estimating the values of its parameters using nonlinear regression. In regression R2 values give relationship between two variables (R2 = coefficient of determination), but it works only for linear, while in case of non- linear it gives misleading information so the most appropriate measure of fitting is the residual sum of squares (RSS) denoted by S^2 and MAE (mean absolute error) and AIC are 2 other measure of fitting of the model. The residual sum of squares essentially measures the variation of modelling errors. Because we were interested in foraging success as a basis for seasonal variation and habitat preference, we tested the effects of habitat and season on foraging success by using different models. Model selection was based on Akaike's Information Criterion. Akaike's Information Criterion (AICc) corrected for small sample size was used to select all final models. AICc is a second- order variant of AIC, which is used when there are many parameters in relation to the size of the sample. Akaike's general approach allows the best model in the set to be identified, but also allows the rest of the models to be easily ranked.

RESULTS

Prey abundance and food composition

In the current study five types of prey items were recorded in five feeding habitats of *A. grayii* (Table 1.5). Main prey items were Insects (Belostomatidae, *Argyroneta aquatica*, Theatops californiensis Eurymerodesmus), Annelids (*Pheretima posthuma*, Hirudo), Fishes (*Channa punctata*, *Channa striatus*, *Clarias batrachus*, *Punctius chola*, *Chela atpar*, *Labeo rohita*), Amphibians (*Rana tigrina*) and Mollusc (Snail). The length (cm) and weight (gm) of prey consumed by *A.grayii* are varied small to medium sized, the size of fishes consumed by it are *Channa punctata (*2.844 ± 0.62 w, 5.42 ± 0.45), *Channa striatus*, *Clarias batrachus (*3.37 ± 0.37 w, 6 ± 1.02), *Punctius chola (*1.52 ± 0.64w, 3.54 ± 0.67), *Chela atpar (1.9* ± 0.80w, 4.56 ± 0.58), *Labeo rohita (2.27* ± 0.44w, 6.06 ± 0.55). Food composition of *Ardeola grayii* varied in habitats and was given in Table (1.4).

Insects were most abundant prey items (48%, 45% and 37% in lake, marshy and pond respectively), while of Fishes were second most abundant (27%, 25% and 17% in pond, lake and marshy area respectively) in feeding habitats of *A. grayii*. Annelids (17%) and Molluscs (9%) were highest in marshy area followed by pond (12% and 10%) and lake (10% and 7%). In Agriculture field, Insects were abundant prey items in Monsoon (36.01%), Winter (69.03%), and Summer (37.64%), followed by Fishes (17.65%, 11.67%, and 16.85% respectively), Amphibians (15.47%, 7.1%, and 6.15%), Annelids (22.03%, 4.06%, and 14.56%), and Mollusc least present in three seasons.

In case of Grassland mainly Insects were present, so in this study only Insects counted as abundant prey for all three seasons, Monsoon (35.06%), Winter (27.80%), and Summer (37.13%).

Prey abundance varies seasonally. Insects (34.58±12.37), Fishes (20.55±6.54), Amphibians (13.08±7.31), Annelids (14.58±8.01) and Molluscs (8.04±3.19) were most abundant in Monsoon in all three habitats, while lowest prey abundance was in Winter (Table 1.5). There were statistically significant differences in prey abundance of pond in three different seasons ($p < 0.05$, Table 1.5). Insects ($F = 6.03$, $p < 0.05$), Fishes ($F = 6.41$, $p < 0.05$) and Molluscs ($F = 3.64$, $p < 0.05$) differed statistically significant for all three seasons in Lake (Table 1.5), but in Marshy area only Fishes ($F = 4.53$, $p < 0.05$) and Annelids ($F = 5.29$, $p < 0.05$) differed seasonally (Table 1.5).

In Agriculture field Insects were most abundant in Monsoon, but for Fishes data was statistically significant ($p < 0.05$, $F = 10.36$), and for other prey such as Insects, Amphibians, Annelids, and for Mollusc data was non-significant, so values of H for Insects ($H = 17.83$), Amphibians ($H = 15.44$), Annelids ($H = 22.90$), and Mollusc ($H = 17.86$). In Grassland only Insects was present in three seasons.

Table 1.4. Food composition of *Ardeola grayii* in different feeding habitats

Food compositio	Lake	Pond	Marshy area	Agriculture field	Grassland
Dragonfly	12	8	5	18	14
Damselfly	8	4	5	2	1
Grasshopper	12	-	17	11	7
Crickets	8	-	-	6	4
Earwigs	-	-	-	8	-
Praying mantis	7	3	1	4	-
Water spider	32	17	-	-	-
Water strider	22	14	-	-	-
Beetles	22	8	17	32	21
Bee	5	1	10	5	8
Caterpillars	12	7	-	18	7
Moth	7	4	5	-	-
Butterflies	-	-	-	-	-
Ants	-	-	-	10	7
Wasps	-	-	-	8	4
Spiders	-	-	-	9	12
Centipedes	8	6	4	2	3
Crustaceans	7	3	12	18	-
Snail	8	6	7	-	-
Leech	12	10	19	-	-
Earthworm	120	88	14	133	-
Small frogs	78	56	12	86	45
Channa	98	34	-	-	-
Mangur	55	54	-	-	-
Sehari	37	42	-	10	-
Sidhari	38	36	-	23	-
Rohu	45	25	-	55	-

Table 1.5 Prey abundance in five different habitats of *Ardeola grayii* at different seasons

Habitats	Prey	Summer	Monsoon	Winter	F/H*	P
	Fishes	14 ± 5.97	19.25 ± 7.32	9.12 ± 2.74	6.445	0.007
	Insects	24.87 ± 8.99	37.37 ± 12.22	20.25 ± 9.05	6.031	0.009
Lake	Amphibians	7.62 ± 12.38	7.5 ± 6.50	2.75 ± 0.70	0.232*	0.63
	Annelids	5.875 ± 6.03	8.12 ± 2.74	3.62 ± 1.18	2.678	0.092
	Molluscs	4.25 ± 2.05	5.12 ± 2.41	2.62 ± 0.74	3.641	0.040
	Fishes	14.75 ± 4.97	19.75 ± 5.47	10.75 ± 2.76	7.826	0.003
	Insects	14.12 ± 3.48	31.12 ± 8.37	15 ± 4.07	22.254	0.001
Pond	Amphibians	5.37 ± 7.58	16 ± 5.09	3.12 ± 1.15	13.448	0.001
	Annelids	4.5 ± 4.37	12.87 ± 7.45	2.5 ± 0.75	10.128*	0.006
	Molluscs	3.12 ± 3.64	9.75 ± 2.60	2.25 ± 1.03	19.107	0.001
	Fishes	7.75 ± 13.57	22.25 ± 6.84	4.75 ± 0.95	4.529	0.044
	Insects	23.5 ±10.27	35.25 ± 16.52	25 ± 9.48	1.047	0.39
Marshy	Amphibians	6.5 ± 11.70	15.75 ± 10.34	2 ± 0	4.891	0.037
	Annelids	6.5 ±11.70	22.75 ± 13.84	4.5 ± 0.57	5.298	0.03
	Molluscs	4.75 ± 3.09	9.25 ± 4.57	5.5 ± 0.57	2.262	0.16
	Fishes	8.58±10.10	14.83±6.17	1.92±2.15	10.36	0.00
	Insects	19.17±9.83	30.25±10.40	11.33±4.77	17.83*	0.00
Agriculture Field	Amphibians	10.59±10.22	13.00±6.80	1.17±2.12	15.44*	0.00
	Annelids	7.42±6.11	18.50±9.72	0.67±1.61	22.90*	0.00
	Molluscs	5.25±2.52	7.42±3.75	1.33±1.77	17.86*	0.00
Grassland	Insects	17.92±7.83	16.92±5.03	13.42±3.87	3.07*	0.21

(* Kruskal wallis H-test)

Foraging attempt and success (Success ratio) of *A. grayii*

Feeding success ratio of *A. grayii* differed seasonally. During summer (Figure 1.1) feeding activity in early hours of observation (7:00h - 8:00 h) gradually increases till (8:00 h) but as time increases feeding activity neither increasing nor decreasing continued in same pattern (9:00-12:00h) for all five habitats, for agriculture field and grassland success ratio peak was maximum at morning hours, stabilized in afternoon, and continued till evening. The feeding activities continued till evening during summer at lake, pond, agriculture field, grassland and marshy area. In marshy area, success ratio graph at lake and pond was in continued in same pattern till evening.

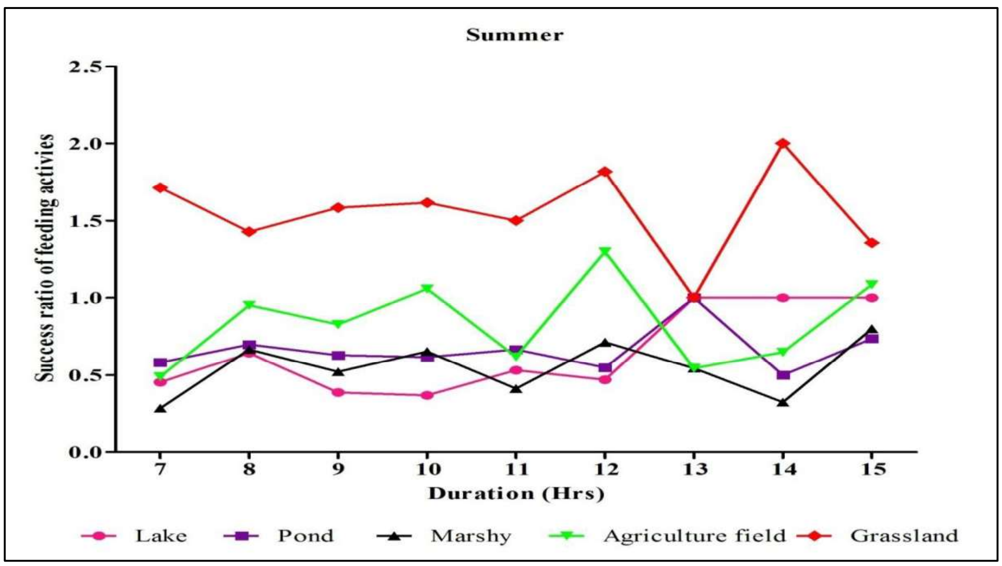

Figure 1.1. Success ratio of *Ardeola grayii* during summer at different habitats

During winter feeding activity in early hours of observation in lake, pond and marshy area was in same pace (Figure 1.2) but in grassland success ratio was attain peak in early morning, 10:00 – 11:00h peak decline, but after 11:00 hours continued in same pace. In winter success ratio was not continued till late evening, feeding continue only in grassland and agriculture field.

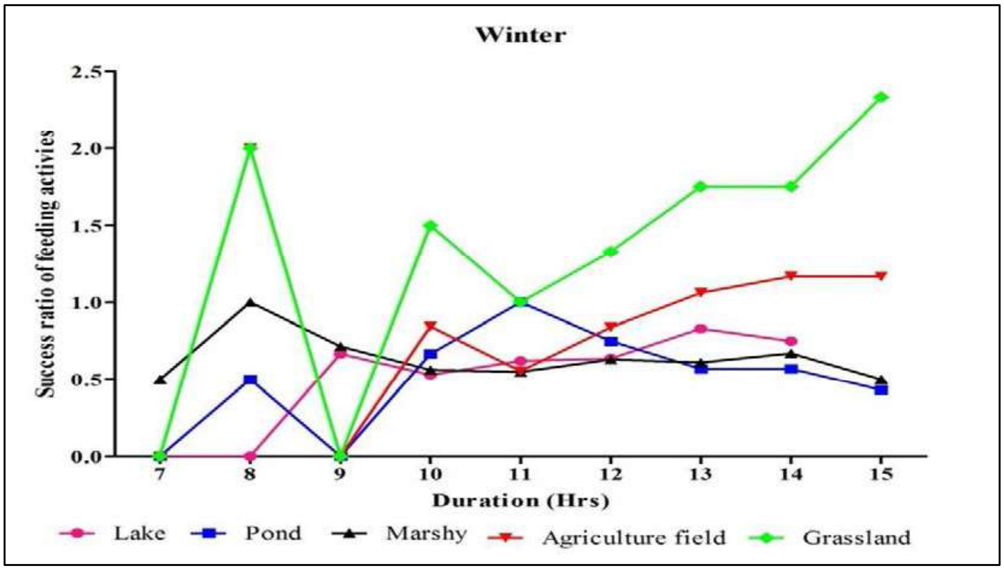

Figure 1.2. Success ratio of *Ardeola grayii* during winter at different habitats

During the monsoon season, the success ratio was higher at morning hours (7:00 - 8:00 h) in grassland and agriculture field. In lake and marshy area, the success ratio was in same pace in morning to evening. But in pond success ratio reached a peak at 10:00 hours and second peak at 12:00 hours at afternoon, after that it was stabilized till evening (Figure 1.3).

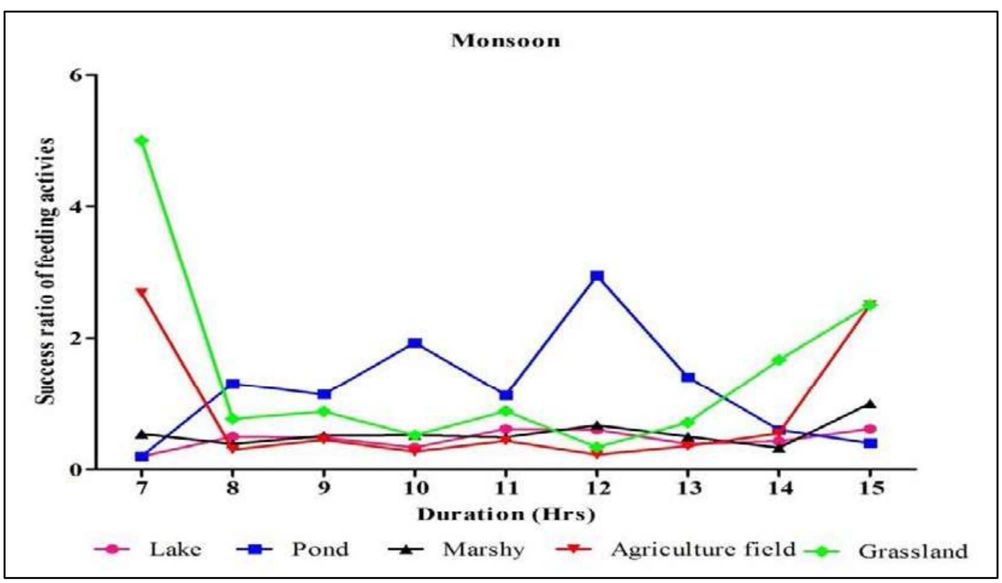

Figure 1.3 Success ratio of *Ardeola grayii* during monsoon at different habitats.

Regression analysis through curve estimation to see feeding pattern of *A.grayii* in three habitats (Lake, Marshy area and Pond).

Out of 11 models (Table 1.6) applied to identify the best fit model for the attempt and successful relationship of *A. grayii*, the best fit models were cubic, exponential, growth and power. The exponential model was found the best fit in monsoon and winter for lake and pond, and marshy land in winter. Initially, success ratio increased steeply and grows until it approaches stability attaining the asymptote.

The growth model (Table 1.6) was found to best fit model in summer season for marshy land and pond. In growth model curve initial point of curve was lower, and with time foraging attempt and success of pond heron increasing and after a certain point, it stabilized. Growth curve represents that in mid of summer season, due to rainy started there was abundant prey available so the peak of the graph was reached a maximum in mid-point. The cubic model (Table 1.6) was found to best fit model in summer season for the lake. In cubic curve, it represents a gradual increase (7:00 - 10:00 h) in foraging attempt and success, in mid of day (11:00 - 1:00 h) curve was in stationary phase and after 13:00 h there was exponential growth. Power model was found to best fit model in monsoon season in marshy land, in power curve there was a proportionate increase in both variables (foraging attempt and success).

Table 1.6. Seasonal variation in feeding frequency of *Ardeola grayii* in different habitats and seasons

Habitat	Seasons	Model	S^2
	Summer	Cubic	1.273
Lake	Monsoon	Exponential	5.439
	Winter	Exponential	2.428
	Summer	Growth	2.084
Pond	Monsoon	Exponential	3.240
	Winter	Exponential	0.504
	Summer	Growth	1.114
Marshy land	Monsoon	Power	5.870
	Winter	Exponential	3.253

Foraging success with reference to prey abundance

The foraging success of was highest in pond (33.60 ± 29.30) followed by lake (31.87 ± 30.85) agriculture field (12.07 ± 6.52), marshy area (11.13 ± 11.61), and grassland (9.73 ± 6.49). Foraging success also differ seasonally, it was observed that foraging success of *A. grayii* maximum in monsoon and lowest in winter in all five habitats (Table 1.7). The current study revealed that foraging success of *A. grayii* differed significantly for fishes ($F = 9.82$, $p < 0.05$), insects ($F = 27.82$, $p < 0.05$), amphibians ($H = 17.28$, $p < 0.05$), annelida ($H = 13.06$, $p < 0.05$), and mollusc ($H = 8.32$, $p < 0.05$) in ponds, while in lake fishes ($F = 5.07$, $p < 0.05$) and insects ($F = 4.15$, $p < 0.05$) and in marshy fishes ($F = 4.03$, $p < 0.05$) and annelids ($F = 5.39$, $p < 0.05$) differed significantly (Table 1.7). In agriculture field foraging success was differed significantly for fishes ($H = 16.31$, $p<0.05$), insects ($H = 16.01$, $p<0.05$), amphibians ($H= 16.56$, $p<0.05$), success for annelids varied, in winter there was no foraging success for annelids, and for mollusc ($F= 5.31$, $p<0.05$).

In grassland mostly insects were present so success for insects was similar in three seasons so there was no variation.

Table 1.7. Foraging success of *Ardeola grayii* at different habitats and seasons

Habitats	Prey	Summer	Monsoon	Winter	F/H*	P
	Fishes	4.75 ± 3.01	7.25 ± 3.53	2.75 ± 1.58	5.068	0.016
	Insects	9.75 ± 3.10	13.37 ± 4.56	8.12 ± 3.35	4.153	0.03
Lake	Amphibians	1.87 ± 2.47	2.62 ± 3.12	1 ± 0	3.844 *	0.146
	Annelida	1.87 ± 1.80	1.87 ± 1.12	1 ± 0	4.519*	0.104
	Mollusca	1.12 ± 0.35	1.37 ± 0.74	1 ± 0	2.277*	0.32
	Fishes	2.75 ± 4.85	7.75 ± 3.59	1 ± 0	4.034	0.05
Marshy	Insects	8.75 ± 4.78	13 ± 5.71	9.5 ± 4.79	0.786	0.485
	Amphibians	2.5 ± 4.35	5.25 ± 3.77	1 ± 0	3.775	0.065
	Annelida	2.25 ± 3.82	8.25 ± 5.18	1 ± 0	5.394	0.029
	Mollusca	1 ± 0.81	1.5 ± 1	1 ± 0	1.114*	0.573
	Fishes	6.5 ± 2.87	9.25 ± 2.91	3.62 ± 1.59	9.818	0.001
	Insects	4.87 ± 1.95	13.87 ± 3.52	5.12 ± 2.53	27.823	0.001
Pond	Amphibians	1.87 ± 2.47	5.37 ± 3.20	1 ± 0/	17.285*	0.001
	Annelida	1.62 ± 1.40	5 ± 4.10	1 ± 0	13.057*	0.001
	Mollusca	1.6 ± 1.34	2.37 ± 1.50	1 ± 0	8.316*	0.016
	Fishes	2.00±2.5	4.42±2.46	0.25±0.45	16.31*	0.00
	Insects	7.08±4.52	9.00±2.21	3.00±1.53	16.01*	0.00
Agriculture Field	Amphibians	1.42±1.62	2.42±1.37	0.17±0.38	16.56*	0.00
	Annelida	0.75±0.75	2.33±1.61	-	-	-
	Mollusca	1.00±0.42	1.08±0.66	0.42±0.51	5.31	0.01
Grassland	Insects	4.33±2.46	5.50±3.52	3.75±1.86	1.17*	0.55

Feeding behaviour of *A. grayii*

In the current study, nine types of feeding behaviour of *A. grayii* recorded in different seasons and habitats: stand and wait (SW), walk slowly (WS), walk quickly (WQ), crouched position (CR), diving (DIV), bill dipping (BD), neck movement (NM), hopping (HOP) and probing (PROB). We found that stand and wait was frequently used behaviour followed by walk slowly, walk quickly, crouched position, diving, bill dipping, neck movement, hopping and probing at different habitats (Table 1.8). *Ardeola grayii* did not used bill dipping in marshy and probing in pond. Stand and wait (SW) ($F = 5.25$, $p < 0.05$) and bill dipping (BD) ($F = 3.18$, $p < 0.05$) statistically differed in all three habitats (Table 1.8), while WS, WQ, CR, DIV, BD, NM, HOP and PROB showed no significant differences in lake, pond and marshy area ($p > 0.05$, Table 1.8).

In lake, *A. grayii* showed significant differences in hopping ($F = 20.63$, $p < 0.05$) and probing ($F = 14.54$, $p < 0.05$) for three season, in all three season, hopping was least used feeding techniques in winter (1 ± 0), followed by summer (2.75 ± 1.70), and monsoon (6.75 ± 2.06), probing was used only in summer (2.75 ± 1.70), and monsoon (6.75 ± 2.06). In marshy, walk slowly ($F = 4.96$, $p < 0.05$), neck movement ($H = 8.89$, $p < 0.05$), hopping ($H = 7.85$, $p < 0.05$), and probing ($F = 5.54$, $p < 0.05$) differed significantly in three seasons. In marshy area, diving was used in monsoon season not in other two seasons. In pond, stand and wait ($F = 4.58$, $p < 0.05$), diving ($H = 6.09$, $p < 0.05$), and bill dipping ($H = 9.35$, $p < 0.05$) differed significantly in all three season. In agriculture field, *A. grayii* showed non-significant differences in all feeding techniques in three seasons, feeding techniques used were SW ($H = 1.61$, $P<0.05$), WS ($H = 18.64$, $P<0.05$), WQ ($H =$

15.14, P<0.05), CR (H = 5.98, P = 0.05), DIV (H = 0.28, P>0.05), and FC (H = 1.80, P>0.05) (fly while catch). The most common techniques were stand and wait and least was fly catching.

In grassland, five techniques used by *A. grayii* , SW (H = 7.02, P<0.05), WS (H = 17.92, P<0.05), WQ (H = 18.71, P<0.05), NM (H = 6.12, P<0.05), and FC (data was constant so test not applicable).

Overall results showed that in all five habitats and seasons, stand and wait was dominant feeding techniques used by *A. grayii*, these were depending on type of prey and structure of habitats.

Table 1.8. Seasonal variation in feeding behaviour of *Ardeola grayii* at different habitats and seasons

Habitats	Behaviour	Seasons			F/H*	p
		Summer	Monsoon	Winter		
Lake	SW	29.75 ± 2.06	35.50 ± 9.846	37.25 ± 5.56	1.39	0.29
	WS	6.25 ± 3.30	8.50 ± 3.10	8.50 ± 1.29	0.91	0.38
	WQ	4.25 ± 1.25	6.25 ± 2.36	5.75 ± 2.21	1.07	0.38
	CR	2 ± 0.81	2 ± 0.81	3 ± 1.63	1	0.40
	DIV	4 ± 2.16	4.75 ± 1.70	3.25 ± 1.70	2.44	0.10
	BD	3.50 ± 3.10	5.50 ± 0.57	3.25 ± 1.70	1.41	0.29
	NM	11.75 ± 10.93	12 ± 11.34	3.50 ± 3.10	1	0.37
	HOP	2.50 ± 1.29	6.75 ± 1.70	1.25 ± 0.50	20.63	0.001
	PROB	2.75 ± 1.70	6.75 ± 2.06	1 ± 0	14.54	0.002
	SW	38 ± 6.37	31 ± 4.54	39.25 ± 7.04	2.13	0.17
Marshy	WS	2.25 ± 2.50	9.0 ± 4.39	7.75 ± 2.36	4.96	0.03
	WQ	2 ± 1.5	6.50 ± 2.88	5 ± 2.16	3.7	0.06
	CR	2.25 ± 1.89	2.50 ± 1.91	2.25 ± 1.50	0.02	0.97

	DIV	-	3 ± 1.63	-	-	-
	BD	-	3 ± 1.63	-	-	-
	NM	1 ± 0	1.25 ± 0.50	8.75 ± 4.99	8.89*	0.01
	HOP	1.75 ± 0.95	4 ± 0.81	1.25 ± 0.50	7.85*	0.02
	PROB	1.75 ± 0.95	5.50 ± 2.08	2.75 ± 1.70	5.54	0.02
	SW	34 ± 2.94	27 ± 12.49	14 ± 10.23	4.58	0.04
Pond	WS	8.25 ± 3.86	7.75 ± 4.03	2.5 ± 2.38	3.3	0.08
	WQ	6.50 ± 3.10	4.50 ± 2.64	2 ± 1.41	3.26	0.08
	CR	4.0 ± 2.82	2.50 ± 1.29	1.50 ± 1	1.78	0.22
	DIV	4.25 ± 0.97	2.75 ± 2.21	1.25 ± 0.50	6.09*	0.04
	BD	5.50 ± 1.73	3 ± 2	1 ± 0	9.35*	0.01
	NM	3.75 ± 1.25	6.25 ± 5.96	3.25 ± 1.70	0.77	0.49
	HOP	4 ± 1.82	2 ± 0.81	2.50 ± 1.29	2.29	0.15
Agriculture Field	SW	20.00 ± 5.18	22.66 ± 5.66	22.66 ± 5.66	1.69*	0.44
	WS	4.08 ± 1.78	8.33 ± 1.92	4.5 ± 2.15	18.64*	0.00
	WQ	2.83 ± 1.40	6.25 ± 1.60	5 ± 2.13	15.14*	0.00
	CR	1.75 ± 0.95	4.75 ± 0.95	4.50 ± 1.29	5.98*	0.05
	DIV	2.13 ±0.83	2.5 ± 0.84	1.5 ± 0.70	2.50*	0.28
	FC	1.86 ± 0.90	2.29 ± 1.49	2.43 ± 1.27	1.80*	0.40
Grassland	SW	15.08±9.01	22.33±4.33	16.58±8.75	7.02*	0.03
	WS	2.08±1.97	2.42±3.91	6.08±2.42	17.92*	0.00
	WQ	1.83±1.52	6.00±2.00	4.50±1.73	18.71*	0.00
	NM	4.00±1.87	9.40±4.50	8.00±2.12	6.12*	0.04
	FC	1.60±0.54	1.50±0.70	2.50±0.70	-	-

(***Kruskal - Wallis H-test**

Table 1.9. Feeding behaviour adopted by *Ardeola grayii* at different habitats

Feeding behaviour	Definition	Habitat
Stand and Wait	When Pond heron stands motionless in water or land waiting for prey to approach	L, P, MA, AF, GL
Walk slowly	Pond heron move slowly, just before striking	L, P, MA, AF, GL
Walk quickly	Pond heron walks through shallow water or fields catching prey disturbed by its movement	L, P, MA, AF, GL
Crouched posture	The body is held horizontal to the perch or the water legs are bent, and the head and neck are partially retracted	L, P, MA, AF
Hopping	Pond heron jump into the air and flies a short distance to a potential prey item	L, P, MA
Diving	Head first or by jumping feet first	L, P, MA, AF
Bill dipping	Moves quickly Involves the bill, the toes, and rubbing	L, P, MA, AF, GL
Bill vibration	This behaviour involves rapid opening and closing of Bill	L, P, MA, AF, GL
Neck swaying	Forward and backward movement of neck to obtain a precise estimate of distance and location of object	L, P, MA, AF, GL
Swimming	More commonly alight on the water and feed only for A matter of minutes before taking a flight again	L, P, MA, AF, GL
Fly catching	Using stand and wait behaviour catches flying insects	AF, and GL
Probing	Quickly and repeatedly moves bill into and out of Water or Mud.	L and MA

(L = Lake, P = Pond, MA = Marshy area, AF = Agriculture Field, GL = Grassland)

In above table 1.8 showed different behaviours adopted by *Ardeola grayiii* in different habitats.

DISCUSSION

Prey abundance

In the current study *A. grayii* used lake, pond and marshy area for feeding activity. Insects were the most abundant prey items, while fishes the second most abundant in all three habitats in feeding habitats of *A. grayii*. Fishes and amphibians were highest in ponds while insects, annelids, and molluscs were in the marshy area. All five habitats having different structures therefore availability of prey items also varies (Meganathan and Jeevanandham, 2019). The availability of preys in the lake is rare only during summer, but as soon as rainy start all available preys in lake increases (Pramanik *et al*., 2015). Thus the availability of prey in the lake is maintained every season. In the pond, all prey is not available in all three seasons, their number increases in monsoon decreases in winter. The abundance of preys such as insects, fishes, annelids amphibians and molluscs also varies seasonally and their abundance was highest in monsoon (Gonzalez, 1997), while lowest in winter in all five habitats. Besides, the habitats season also play an important role in the fluctuation of preys in different habitats (Kushlan, 1979; Kumar and Kumar, 2018). *A. grayii* generally chosen marshy during monsoon (Kushlan, 1979; Kushlan, 2011; Maheswaran and Rahmani, 2002), fishes, amphibians, annelids, and aquatic insects begin their reproductive activity during monsoon in marshes (Bates and Ballard, 2014), pond and lake (Bates and Ballard, 2014), which guarantees a relatively high abundance of potential prey for *A. grayii* in these habitats (Seedikkoya, 2012). The marshy area after winter dries rapidly and become inadequate for *A. grayii* feeding site. Agriculture field and grassland as feeding habitats used by it mainly during monsoon and summer season because in that period most of the prey was abundant. These results suggest that prey diversity increased in number during monsoon, so all prey items were present abundantly and in summer and winter insects and fishes mainly present in all habitats.

Foraging success with reference to prey abundance

Foraging success of *A. grayii* depend on many factors such type and size of prey, techniques used by it, structure of habitats, depth of water bodies, variation in seasons, and on availability of prey. In this study, it was observed that this bird generally preferred to feed shallower water depth 0.5- 7cm, similar result was also observed by Jayson and Sivaperuman (2011), and habitat with more vegetation and it generally choose larger feeding area. Its preferred food are fishes and insects, when these prey not present in habitats then it skips to others such as amphibians, annelids and mollusc. *A. grayii* preferred water depth 10-15cm for capturing prey, less vegetation height, preferred size of prey were, for fishes 1-7cm, insects 0.5-5cm, annelids 3-7cm, amphibians 3-5cm, and mollusc 3-8cm, this was also supported by Roshnath (2015) and Porte *et al*. (2018).

In this study foraging success was more with fishes and insects in three seasons and five habitats, increasing order for success on capturing preys were Insects > Fishes > Amphibians > Annelids > Mollusc.

Foraging attempt and success (Success ratio) of *A. grayii*

Prey abundance, the structure of habitats, variation in seasons determined the success ratio of *A. grayii* **.**In the summer season, due to high temperature all wetlands having low water level, so very few prey available but during monsoon, prey availability increases, as a result graph plotted between attempt and success showed more fluctuation in peak height than other seasons. It was also observed that *A. grayii* feeding activity continued till late evening in summer (Porte *et al*. 2018).

The success ratio of *A. grayii* was high during monsoon due to availability of various prey items such as, amphibians, insects, Annelida, and fishes. In monsoon, foraging success did not only depend on prey abundance but also water level, if the water level was not approachable to *A. grayii*, it did not feed, as the water level goes down, it started to feed. In winter, as temperature decreases very few preys available for capture so *A. grayii* success ratio decreases.

Some model used for feeding pattern of *A. grayii* in three feeding habitats.

Exponential curve showed feeding pattern of *A. grayii* in winter and monsoon season in all three habitats, exponential curve pattern shows in starting of winter foraging attempt and success was increased but as temperature decreases, availability of prey decreased as a result foraging frequency decreased, in case of monsoon availability of prey abundant but due level of water was high so *A. grayii* was not easily approached to prey so initially there was low frequency of feeding as water level goes down feeding frequency again raised.

Growth curve represents the feeding pattern of *A. grayii* in summer season in both pond and marshy land. in such type of pattern of curve was due to availability of prey in habitats, in summer, starting of summer season there is starting of breeding period of *A. grayii*, most of the time it was observed that busy in searching suitable place for nest construction, so there feeding frequency was low, but as that part of breeding over its feeding frequency gradually increases (Seedikkoya, 2012). The cubic curve showed the feeding pattern of *A. grayii* in the lake during the summer season. The cubic curve showed the initial frequency of feeding was slow after sometimes itincreases, in summer availability of prey was not abundant as monsoon season, but the requirement of energy for A. grayii was high due to breeding period so the curve was increasing.

Power curve represents the feeding pattern of *A. grayii* in marshy land during monsoon season. Marshy land provides a temporary (Bates and Ballard, 2014), habitat for feeding. In the monsoon season, most of the fishes, amphibians and aquatic insects begin their reproductive activity during monsoon season, which resultant a relatively high abundance of potential prey for *A. grayii* for these habitats. Power curve showed a proportionate growth of foraging attempt and success because there was abundant prey available, it had not to wait for longer for capturing.

Feeding behaviour of *A. grayii*

Members of family *A. grayii* varied their feeding techniques according to habitats (Meganathan and Jeevanandham, 2019) as well as several environmental factors such as water depth (Bates and Ballard, 2014), the height of vegetation (Hafner *et al*., 1982; Calvin, 1983), and availability of prey (Bates and Ballard, 2014). *A. grayii* mainly used the stand and wait for technique in all five habitats. However, in addition to stand and wait *A. grayii* also adopted the walking slowly, similar techniques also adopted by Great egrets and Little egrets (Hafner *et al*. 1993; Samantha *et al*. 2011; Roshnath, 2015). *A. grayii* used walk slowly for capturing sedentary or slow-moving prey in shallow water, marshy and vegetated area (Papakostas *et al*. 2005; Samantha *et al*. 2011; Porte *et al*. 2018).

Stand and wait is better for capturing large prey in deep water or for finding hidden prey (Dimalexis *et al.* 1997). Crouched position, hopping and probing was very least preferred techniques by *A. grayii* for foraging in three habitats. Stand and wait technique differ in ponds, lakes and marshy area, while no other techniques differ according to habitats. *A. grayii* did not use bill dipping in marshy and probing in the pond. Feeding techniques of *A. grayii* not much varied with seasons, only HOP and PROB varied in lake and marshy while DIV and BD in ponds.

In pond stand and wait, diving and bill dipping differ significantly, because, in summer near pond, mostly fishes available for foraging, and that time was breeding period for it, so they have to feed oneself, nest construction, rearing young ones, that's why they could not go for longer distance, so they waited for long and during breeding mostly depend on fishes (Roshnath, 2015). Near pond mostly these three techniques (stand and wait, diving and bill dipping) used by pond heron. In agriculture field and grassland frequently used behavior was fly catching and this technique was used to capture flying insects.

Overall, the present study indicates that prey availability, habitat structure, feeding techniques, all depend on variation in season, which ultimately affects feeding activities of *A. grayii.*

CONCLUSION

In this study, it was concluded that *A. grayii* employs different behaviour for feeding, for survival of any prey or predator feeding techniques is play important role in their foraging success, so each species used more than one feeding techniques and this helped in the wading birds to utilise the resource from different habitats. The feeding techniques mostly used in all habitats and seasons was stand and wait, insects were present abundantly in all five habitats, so foraging success for insects were higher than other prey items. But during summer and starting of monsoon fishes was mostly preferred by *A.grayii*. Monsoon season was the favourable season for feeding of herons due to availability of prey, so in all five habitats foraging success was higher in monsoon. In this study it was observed that insects were mostly consumed prey by *A. grayii,* so it plays a significant role in controlling the population of insects and other invertebrates on which they fed and supports the farmers. However, the rapid urbanization and development of the area for living and other human purposes may create a threat to these birds by the destruction of natural habitats through anthropogenic activities. Thus, if any disturbances occur in habitats of *A. grayii* its feeding activity will affect, resulted in their breeding activity will hamper finally decrease in their populations. All organism living on this earth have equal right to live, and every organism directly or indirectly interconnected to each other and having important role in balancing of ecosystem. If any one of them disturb, the whole system will be affected. So every single species should be take care and conserved.

Figure 1.4 Feeding habitats of *Ardeola grayii* (Study sites 1-6)

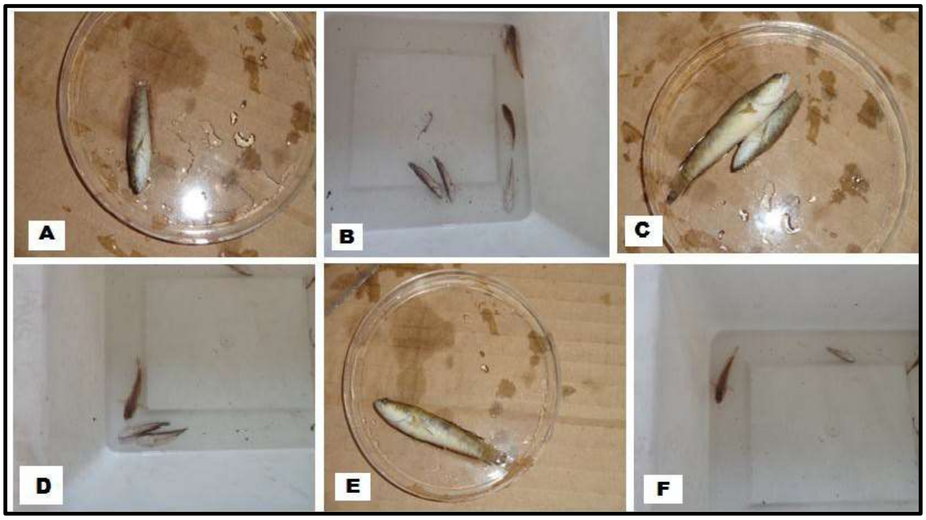

Figure 1.5 Sample of fishes collected for measurement of length and weight from feeding habitats of *Ardeola grayii*

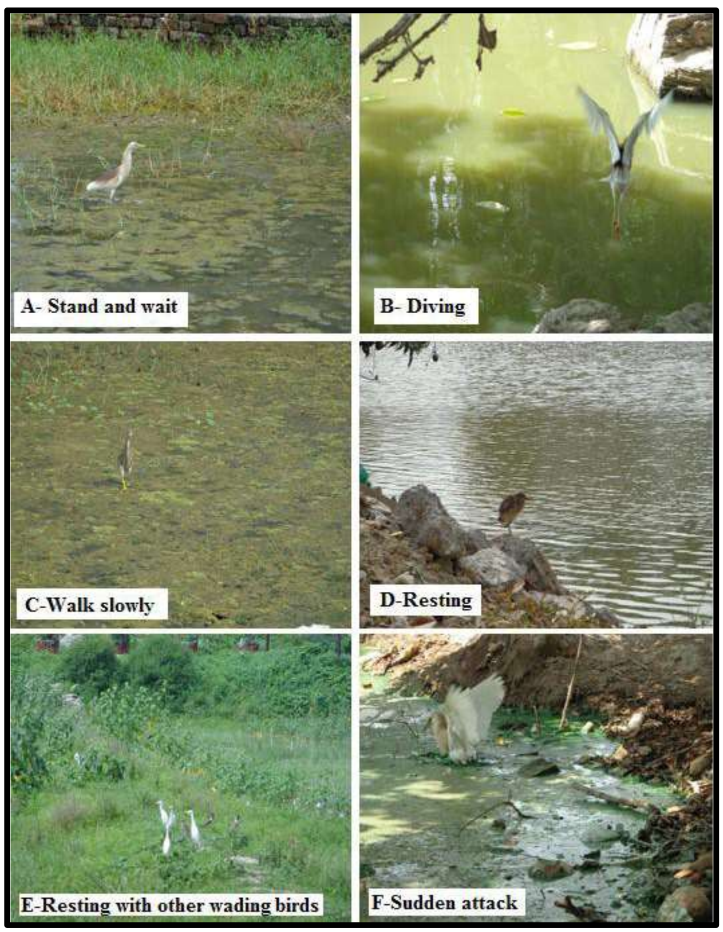

Figure 1.6 Behaviour of *Ardeola grayii* in different habitats (A- Stand and wait, B- Diving, C- Walk slowly, D- Resting, E- Resting with other Wading birds, F- Sudden attack)

Chapter-II

The pollutants of ponds and wetlands and their effects on behaviour of *Ardeola grayii*

CHAPTER-II The pollutants of ponds and wetlands and their effects on behaviour of Ardeola grayii

INTRODUCTION

Natural treasures are the important wealth of our country. Water is one of them. It is most important resource in shaping the land and regulating the climate. Water resources are of critical importance to both natural and human development. It is essential for agriculture, industry and human existence. The healthy aquatic ecosystem is dependent on the physico-chemical and biological characteristics. The quality of water in any ecosystem provides significant information about the available resources for cope with life in that ecosystem. Wetlands are the major players in maintaining water cycle and habitats for all wading birds. It provides breeding sites and resting ground for many birds and also inhibits aquatic biota. Wetlands are also used by human long ago as aquaculture for rearing prawn, fishes. There has been a strong relationship between human activity and disturbance of the aquatic environment (Hodkinson *et al*., 2005). Wading birds and aquatic organisms are dependent for their daily activities on water bodies, and it has the ability to detect, discriminate, and respond to the pollutants and are also sensitive to both beneficial and harmful chemicals. Recent findings have proposed that deterioration of water quality is because of excess acidification and the presence of nitrogen, phosphorus, heavy metals, organic toxicants, and pesticides (Bloxham *et al*., 1999). Pollutants introduced to the environment have impact on ecosystems, and is found in the whole biosphere. The maintenance of healthy aquatic ecosystem is dependent on the physico-chemical properties of water and biological diversity. One of the most important and easily detectable qualities of water is pH, its value of water is an important indication of its quality, and it is dependent on carbon-dioxide, carbonate-bicarbonate equilibrium. Other physico-chemical parameters such as dissolved oxygen, biological oxygen demand, chemical oxygen demand, chloride, total hardness and alkalinity also indicator of healthy aquatic ecosystem. Pollution in wetlands is not only declining the water quality, but also create pressure on the aquatic biota and animals openly or obliquely but, also create a reduction in the range of several avian species, leading the biodiversity in wetlands decline.

Lucknow the capital of Uttar Pradesh situated on the bank of the river Gomati, which is the habitat of faunal diversity. Unfortunately, due to intense colonization, discharge of harmful industrial wastes, cutting of trees, conversion of agriculture field into buildings, its air and water quality are not healthy and sometime air quality index reached to 493, water reservoirs has severely impacted. *Ardeola grayii* can be easily seen in both urban and rural area but due to decreasing water body in urban area of Lucknow, its population is decreasing rapidly.\

In the present study*, Ardeola grayii* is used as indicator for habitat quality. Birds are considered a good bio-indicator on habitat quality and the effectiveness of ecosystem, providing services to ecosystem and humans, as well as a good indicator of pollution and biodiversity. They are one of the connecting links of the food chain in the ecosystem and having important role in their habitats. There is reciprocal relationship between environment and birds. Environment provide essential factors such as resting, breeding, feeding, ground for their survival, while birds contribute direct and indirect role in maintaining environment or modifying certain environmental components (Reid, 1991; Block and Brennan, 1993). In addition, birds are considered as an excellent communication means to raise awareness of biodiversity issues in a way that many organisms cannot (Gregory and Strien, 2010).

Water pollution is becoming a big problem for biodiversity. The main source of water pollution is industrial waste, domestic waste, sewage wastage which directly flow in water body and acid rain is also contribute in water pollution which cause deposition of heavy metals in water bodies (Obasohan *et al*., 2008). Fresh water is a source for the development of civilizations but due to pollution there is severe threat to natural fresh water reservoir (Benjamin *et al*., 1996). The impairment of water quality due to introduction of pollutants is a problem faced by most industrial cities around the world. Rapid urbanization and industrialization with improper environmental planning often lead to discharge of industrial and sewage effluents into wetlands. The wetlands have a complex and fragile ecosystem, as they do not have a self-cleaning ability and therefore readily accumulate pollutants.

Heavy metals entering the water body would be adsorbed in sediments, and subsequently might migrate as a result of exchanges between water, sediment, and biota, through biological and chemical process. Heavy metals do not degrade in water but are generally not found in high concentrations, primarily due to deposition in sediments but also because of uptake by aquatic organisms. Birds are exposed to heavy metals through air, water and their food. Once a metal has entered the body it can be stored or accumulated, or be excreted (Dauwe *et al*., 1999). Heavy metals impact on metabolic and reproductive ecology of birds. Water quality influences the availability and accessibility of prey items to various aquatic predators. The water quality is important in water bird habitat assessment because a host of interacting physical and chemical factors can influence the level of primary productivity in aquatic systems and thus influence the trophic structure and total biomass throughout the aquatic food web (Wetzel, 2001). The physico-chemical characteristics of the water largely determine the waterbird community of wetland habitats, primarily by their direct and indirect impact on the availability and abundance of the birds prey (Nagarajan and Thiyagesan, 1996). The physico-chemical environment can also directly and indirectly affect waterbirds daily activities. In a direct way, for example, different species of shorebird are constrained morphologically to forage at specific water depths (Safran and Isola, 1997). Indirectly, however, physico-chemical variables such as salinity and acidity affect the distribution and richness of benthic invertebrates (Courtney and Clements, 1998; Leland and Fend, 1998; McRae *et al*., 1998), which in turn can affect the feeding ecology of waterbirds.

So due to all these pollutants either wastage dumped in water or metals present in trophic levels causes variations in such as pH, biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), Chloride, hardness, calcium carbonate, magnesium carbonate, alkalinity in the water bodies directly or indirectly disturb the biodiversity (Odum, 1971).

The aim of the present study was to discuss physico-chemical parameters and heavy metals present in water, sample collected from feeding ground of *A. grayii.* This study was divided into two parts, in first part physico-chemical parameters and heavy metals of water analyzed in highly contaminated and less contaminated feeding habitats of *A. grayii* and in second part in highly contaminated and less contaminated area, it's behaviour was observed and compared to normal behaviour.

MATERIALS AND METHODS

Study area

Lucknow is the capital of Uttar Pradesh situated 123 meter above sea level, geographic coordinates of Lucknow is 26.8470 N and 80.9470 E. during study different wetlands covered, and it is divided into five sites viz, S1, S2, S3, S4, and S5. All these sites are used by *A. grayii* as feeding habitats, site 1 (2644’16” N, 8057’18” E) and site 2 (2645’31’’N, 8056’32’’E) are lake but site 1 man-made lake and site 2 natural lake, site 3 (2633’21’’N, 8030’38’’E), site 4 (271’20” N, 8050’7’’E), and site 5 (2645’34’’N, 8051’21’’E) is pond. Lucknow having rich wetlands biodiversity, due to presence of Gomati and its tributaries rivers, but due to local pressure such as increasing population and anthropogenic activities the wetlands are in threat condition.

Parameters for analysis of water quality

In this study some physico-chemical parameters of water such as, pH, biological oxygen demand, dissolved oxygen, chemical oxygen demand, total hardness, magnesium as calcium carbonate and calcium as calcium carbonate, chloride and alkalinity. The values of different physico-chemical parameters of the water of different area of Lucknow (upto 50 km) from all the samplings points during Sep 2016 to Feb 2019 are given in Table 2.2, 2.3, and 2.4. The values are given in mean ± SD for observation from all the 5 sampling points.

Heavy metals were also analyzed through water samples, metals analyzed were, Cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), manganese (Mn), and zinc (Zn). All these parameters were followed by CPCB (2006, 2008), and APHA (2005).

Sample collection and preservation

In order to analyze physico-chemical and heavy metals in water, samples were collected in twice a month in replicates in urban and rural area of Lucknow from September 2016 to February 2019, in pre-cleaned polyethylene bottles. The surface water samples were thoroughly filtered through cellulose nitrate filter paper to eliminate suspended solids and stored in plastic bottles with one-liter capacity. For analysis of pH, buffer solutions are necessary to standardize the pH meter: Buffer solutions are used in the laboratory for pH = 4, 7, and 9.

DO is determined in a sample collected in a DO bottle using a DO sampler. The DO in the sample is fixed immediately after collection, using chemical reagents manganous sulphate solution were added to fix DO. Samples for BOD analysis, it is stored at a temperature below 4℃ and in the dark as soon as possible after sampling. In the field BOD bottles are means placing in an insulated cool box together with ice or cold packs. Once in the laboratory, samples are transferred in a refrigerator.

The BOD5 was computed by subtracting the DO after five days' incubation from the DO measured on collection of samples at point in mg/L.

$$\mathbf{BOD5 = DOi - DOf}$$

Here,

DO_i = initial DO of diluted sample

DO_f = final DO of diluted sample

Samples collected for chemical oxygen demand (COD) analysis is not analyzed on the day of collection so it was preserved below pH 2 by addition of concentrated sulphuric acid.

The COD was calculated by following formula,

COD (mg/l) = (V_b – V_s) ×M × 16000/ ml of sample Here,

V_b = volume of blank

V_s = volume of sample M = molarity

Samples which are to be analyzed for the presence of metals are acidified to below pH 2 with concentrated nitric acid. These samples can then be kept up to six months before they need to be analyzed, but for mercury determinations, it was carried out within five weeks.

Appropriate drift blank was taken before the analysis of samples. The working wave length for the heavy metals are 279.5 nm for Mn, 213.9 nm for Zn, 228.8 nm for Cd, 357.9 nm for Cr, 217 nm for Pb, and 253.7nm for Hg.

Instrument used

pH was analyzed through pH meter in electrometric method, there is no specific unit for it.

Dissolved oxygen analysed in Winkler's method, unit of DO is mg/L

Biological oxygen demand is analysed to see dissolved oxygen consumption in 3 days at 27℃, unit of BOD is mg/L

Chemical oxygen demand analysed through potassium dichromate method, unit of COD is mg/L

Total hardness is measured through complexometric (EDTA) titration, unit of hardness is mg/L

Chloride is analyzed through argento-metric titration, unit of chloride is mg/L

Alkalinity analyzed through acid-base titration, unit of alkalinity is mg/L

Mercury is analyzed through cold vapour AAS, and other metals such as, Cd, Pb, Cr, Mn, and Zn analyzed through AAS, measuring unit for all metals is µg/L.

Statistical analysis

To identify the variation in physico-chemical parameters in five sites, three year (2016-2018), (2017-2018), and (2018-2019) and three season (Summer, Winter, and Monsoon), every month two samples were taken for each sites, average was calculated after three measurement, after that, all data for every year pooled and then checked normality by using SPSS and then on the basis of significant and non-significant data tests one way ANOVA (when $p < 0.05$), and Kruskal - Wallis (when $p > 0.05$) used to see variation in concentration of metals as well as physico-chemical parameters.

Table 2.1. Permissible limit of Physico-chemical parameters and Heavy metals of water

Parameters	CPCB
pH	6.5-8.5
BOD	3 – 6 mg/L
COD	>10mg/L
DO	<5 mg/L
Chloride	250 mg/L
Total hardness	300 mg/L
Calcium as calcium carbonate	200 mg/L
Magnesium as magnesium carbonate	100 mg/L
Alkalinity	200 mg/L
Cd	No relaxation
Cr	No relaxation
Pb	No relaxation
Hg	No relaxation
Mn	0.05
Zn	15.0

In order to analyze physico-chemical and heavy metals in wetlands, water samples were collected in twice a month in replicates in urban and rural area of Lucknow from September 2016 to February 2019, in pre-cleaned polyethylene bottles. Water samples were analyzed for heavy metals (Pb, Cd, Cr, Hg, Zn and Mn) in Atomic Absorption Spectrophotometer. The surface water samples were thoroughly filtered through cellulose nitrate filter paper to eliminate suspended solids and stored in plastic bottles with one-liter capacity. For the measurement of dissolved oxygen (DO) and biological oxygen demand (BOD), separate 300 ml clean glass stopper BOD bottles were used for sample collection (standard volumetric Winkler's method). For metal analysis, 5 ml nitric acid was immediately added after collecting the samples, for heavy metal samples were collected separately. Digested samples were placed in pre-washed polyethylene bottle, various standards of heavy metals were prepared from certified standard stock solution (ppm) by using double distilled water. These standards were used to obtain calibration curve on Atomic Absorption Spectrophotometer. Effect of pollutants and changes in physico-chemical parameters of water on *A. grayii* behaviour was also observed. All parameters and procedures followed from CPCB 2006, 2008 and APHA 2005. Normality of data were analyzed through SPSS (version 21.0) for every year data, mean ±SD values were taken, and graph created by Microsoft Excel.

RESULTS

Analysis of Physico-chemical parameters:

The physico-chemical environment of water functions in many ways and employs the influences upon biotic components, thus, giving a picture of the environmental suitability of water to maintain life (Kumar and Singh, 2002). Temperature affects various chemical and biological reactions taking place in water and aquatic organisms and depends upon the season, time of sampling and also upon the temperature of effluent which is being added into the river (Shrivastava and Patil, 2002).

Main sources of pollutants in study area

Lucknow is developing fast with rapid urbanization, its 46 percent of water bodies is shrinking due to dumping of waste and sewage. Lake and pond are helpful in accumulation of rainwater and enhance groundwater level, and inhabitants of many local and migratory birds, but gradually condition is deteriorated. It was observed that there is declining in number of waterbirds and wetlands in study period (2016 - 2019). The main sources of pollutants in studies area are; unmanaged industrial operations, sewage, kitchen, hospital discharge, agriculture and urban runoff, solid waste and garbage tied in polythene bags floated on water surface, organic and inorganic pollutants, dumping of wastage in water bodies.

Sources of heavy metals in water body are chemicals used in painting purposes, tanneries, steel industries, fly ash, kitchen appliances, surgical instruments, steel alloys, automobile batteries, these are common sources of heavy metals, their impact on human as well as wading birds.

pH

In any water body, all chemical and biological reactions directly depend on the pH of the system. Water pH is influenced by geology of catchment area and buffering capacity of water. The mean variations (Mean ± SD) of the water physico-chemical parameters for the three-year study periods are given in the Tables 2.2, 2.3 and 2.4, respectively. In this study 3 years observation was done, in 2016-2017 in all five habitats pH was ranges between 6.3 – 6.9, too much fluctuation in pH are stressful and can even be lethal to aquatic organisms, which may circulate in a food chain. Levels of pH too high (> 9) or too low (< 5) can kill aquatic life (Younos, 2007).\

Biochemical Oxygen demand (BOD)

Biochemical Oxygen demand determines the amount of oxygen required for biological oxidation of organic matter with the help of microbial activities. In the present study the value of biochemical oxygen demand ranged between 5.06 to 29.03 mg/L (Table 2.2).

Chemical oxygen demand (COD)

Chemical oxygen demand (COD) determines the amount of oxygen required for chemical oxidation of most organic matter and oxidizable inorganic substances with the help of strong chemical oxidant. High COD indicates the presence of all forms of organic matter, both biodegradable and non-biodegradable, and hence the degree of pollution in waters. In the present study the value of COD ranged between 14.69 to 27.82 mg/L which show its high pollution status in S-2 and S-5 sites due to input of domestic drains, dumped plastics, hospitalized wastages, the use of soap and detergents in bathing and washing purposes.

Dissolved oxygen (DO)

Dissolved oxygen for all five habitats was 4.3 – 7.36 mg/L, DO > 5 mg/L is considered favourable for growth and activity of most aquatic organisms; DO < 3 mg/L is stressful to most aquatic organisms, while DO < 2 mg/L does not support fish life (USEPA, 1999). In this study site 2 was having oxygen deficit than others four, site 2 was a very large lake but due to increasing population it was constantly dwindling and filling with garbage.

Alkalinity in this study was ranges from 103.03 – 361.7 mg/L. the lake can be categorized as nutrient rich water body and highly productive on the basis of total alkalinity. If any water body have > 20mg/L alkalinity is good for community production, it is also the measure of buffering capacity of water. It is important to assess the alkalinity of water bodies to determine the ability of neutralizing the acidic pollution of water from rainfall of waste water. Total alkalinity is measured on the basis of some components such as bicarbonate, carbonate and hydroxide. According to Durrani (1993) withdrawal of CO_2 from the bicarbonates for photosynthesis by algae may increase total alkalinity. In this study S-2 and S-3 was having high alkalinity, S-3 was near to temple so by religious activities, people feed fish that causes pond become nutrients rich.

Chloride

Chloride is found widely distributed in nature in the form of salts of sodium, potassium and calcium. The chloride status in water is indicative of pollution, especially of animal origin. In the present study chloride concentration was found ranging between 22.10 to 59.8 mg/L (Table 2.2). Site -2 was having more chloride level due to large amount of organic matter, waste of animals dumped in lake.

Total hardness

Total hardness of water is the measure of alkaline earth elements such as calcium and magnesium in an aquatic body along with other ions such as aluminium, iron, manganese, strontium, zinc, and hydrogen ions. In this study the total hardness of water range was 137.7 to 311.36 mg/L in study area. Site 2 had highest 311.36 mg/L total hardness in water.

Calcium as calcium carbonates and Mg as magnesium carbonate

As the content of Ca and Mg in water increases, the content of hardness also shoots up (Shrivastava and Patil, 2002). The average value of calcium hardness for the study period was 29.6-55.4mg/L, whereas the average value for Mg^{2+} recorded was 22.1-60.7 mg/L for the study period. Calcium and magnesium are the dominant cations in an aquatic body. Higher concentration of calcium and magnesium is due to the dissolution of carbonate minerals in water through rainwater mixing, while a lower concentration is due to increased photosynthetic activity of aquatic organisms (Divya, 2013).

In 2016-2017 pH did not varied significantly for all five sites, but other physico-chemical parameters varied significantly (Table 2.2).

Table 2.2. Variation in Physico-chemical parameters of water at different areas of *Ardeola grayii* during 2016-2017 (all values are given in Mean ± SD)

Physico-chemical Parameters	S1	S2	S3	S4	S5	F/H* (P)
pH	6.3±0.15	6.5±0.3	6.4±0.1	6.9±0.15	6.6±0.37	6.3*(0.17)
BOD	23.76±1.49	29.03±1.00	14.4±0.60	5.06±0.51	24.73±1.45	13.03*(0.01)
COD	23.19±0.45	27.82±0.20	18.38±0.47	14.69±0.37	26.88±0.32	12.83*(0.01)
Chloride	50.1±0.1	59.8±0.62	22.10±0.20	36.2±0.15	31.93±0.20	13.50*(0.00)
Dissolved oxygen	6.3±0.20	4.3±0.20	6±0.1	7.1±0.2	7.36±0.15	13.11*(0.01)
Total hardness calcium as calcium carbonate (mg/l)	165.1±0.1	311.36±1.18	294.6±4.16	144.2±1.08	137.7±8.63	13.50*(0.00)
Calcium as calcium Carbonate	29.6±0.45	46.1±0.1	33±0.78	55.4±0.4	50.63±0.37	13.50*(0.00)
Magnesium as magnesium carbonate	22.1±0.2	47.3±0.20	47.21±0.26	60.7±0.61	32.13±0.90	13.50*(0.00)
Alkalinity	103.03±1.76	361.7±0.60	255.3±1.18	111.46±1.28	323.2±0.26	13.50*(0.00)

In 2017-2018 (Table 2.3) it was observed that, site 2 (7.8 pH) and site 3 (7.23 pH) become slightly alkaline than other three sites. In site 2, DO (4.6 mg/L), BOD (8.9 mg/L) lowest and highest COD (41.8 mg/L) was observed. Total hardness was maximum in site 2 (308.7mg/L) and site 5 (308.7 mg/L), calcium and magnesium was maximum in site 5 (56.5 – 60.9 mg/L). Alkalinity was observed maximum in site 2 (357.5 mg/L) and site5 (319.8 mg/L). Degree of freedom for all these data is 4 (df = 4)

Table. 2.3. Variation in Physico-chemical parameters of water at different areas of *Ardeola grayii* during 2017-2018 (all values are given in Mean ±SD)

Physico-chemical Parameters	S1	S2	S3	S4	S5	F/H* (P)
pH	6.3±0.32	7.8±0.25	7.23±0.15	7±0.1	6.9±0.41	11.38* (0.02)
BOD	27.43±1.06	8.9±0.6	16±0.1	35.1± 0.1	27.7±0.55	12.87* (0.01)
COD	29.7±0.5	41.8±0.5	32.8±0.37	34.43±0.30	32.3±1.10	12.88* (0.01)
Chloride	43.3±1.07	54.16±1.5	20.83±0.6	42.2±0.9	40.7±0.25	12.83* (0.01)
Dissolved oxygen	5.5±1	4.6±0.50	5.03±0.15	6.1±0.35	5.46±0.35	7.56* (0.10)
Total hardness calcium as calcium carbonate (mg/l)	167.9±0.05	308.7±2.3	299.8±0.72	167.83±0.40	308.7±2.3	12.90* (0.01)
Calcium as calcium Carbonate	28.3±0.46	44.83±0.5	33.1±0.3	32±0.69	60.9±0.7	13.54* (0.00)
Magnesium as magnesium carbonate	21.34±0.9	47.16±1.35	47.3±0.26	44.7±0.55	56.5±0.39	12.84* (0.01)
Alkalinity	106.7±2.3	357.5±6.5	298.4 ± 2.4	166.43±1.64	319.8±0.66	13.50* (0.00)

(* Kruskal - Wallis H-test)

In 2018-2019, it was observed that (Table 2.4), site 2 (8.1 pH) and site 5 (8.03 pH) was alkaline than other three sites, BOD was minimum in site2 (12.5 mg/L), COD maximum in site2 (60.7 mg/L), and dissolved oxygen minimum in site 2 (3.8 mg/L) and in site 5 (4.56 mg/L). Total hardness of water was maximum in site 2 (333.9 mg/L) and site 5 (351.06 mg/L). degree of freedom for all these sites are df = 4.

Table 2.4. Variation in Physico-chemical parameters of water at different areas of *Ardeola grayii* during 2018-2019 (all values are given in Mean±SD)

Physico-chemical Parameters	**S1**	**S2**	**S3**	**S4**	**S5**	**F/H* (P)**
pH	7.06±0.15	8.1±0.26	7.03±0.15	7.5±0.36	8.03±0.15	11.48* (0.02)
BOD(mg/l) 3 days at 27℃	27.3±1.70	12.5± 0.20	17.2±0.9	43.2±2.74	41.3±1.21	13.03* (0.01)
COD(mg/l)	24.7±0.50	60.7±2.08	33.3±0.9	31.4±4.3	34.3±1.1	11.23* (0.02)
Chloride (mg/l)	44.7 ±0.5	62.1±1.90	22.7±0.5	28.6±0.7	45.3±0.25	13.38* (0.01)
Dissolved oxygen	7.3±0.20	3.8±0.25	6.2±0.15	5.3±0.47	4.56±0.32	13.5* (0.00)
Total hardness as calcium carbonate	155.3±1.01	333.9±11.1	266.8±11.5	176.1±0.86	351.06±5.5	13.52* (0.00)
Calcium as calcium Carbonate	26.4±1.15	45.16±4.3	36.9±0.68	41.6±1.37	61.46±1.5	13.05* (0.01)
Magnesium as Magnesium carbonate	25.27±1.00	50.5±1.70	40.2±1.11	57.9±0.62	51.5±0.6	12.9* (0.01)
Alkalinity	113.7±5.8	322.13±4.3	295.7±4.2	181.9±0.78	311.5±1.22	11.23* (0.01)

(*Kruskal - Wallis H-test)

Seasonal variation in Physico-chemical parameters of water in different study sites

Seasonal variation in physico-chemical characteristics of water determine the abundance of bird's prey. The hydrogen ion concentration (pH) was highest in winter (6.9), lowest in summer (6.6). The highest biological oxygen demand value of (13.66) was found in summer, while lowest in winter (9.13).

The highest dissolved oxygen was found in winter (6.7), and lowest in summer (5). The value of chemical oxygen demand was highest in summer (54), and lowest in winter (24). Chloride was highest in winter (38) and lowest in summer (24). The total alkalinity was maximum in summer (298), and minimum in winter (159.4). total hardness of water was maximum in summer and lowest in winter. The value of physico-chemical parameters for all seasons in five study sites are given below;

Table 2.5. Seasonal variation in Physico-chemical parameters of water in different study sites

Physico-chemical Parameters	Summer	Winter	Monsoon
pH	6.6 ± 0.17	6.9 ± 0.2	6.53 ± 0.51
BOD(mg/l) 3 days at27°C	13.66 ± 11.50	9.13 ± 2.87	10.13 ± 5.53
COD(mg/l)	54 ± 47.50	24 ± 17.10	44.18 ± 18.44
Chloride (mg/l)	24 ± 1.51	38 ± 17.02	34 ± 2.22
Dissolved oxygen	5.66 ± 1.04	6.7 ± 0.98	5.7 ± 0.78
Total hardness as calcium carbonate	255.73 ± 79.1	146.04 ± 8.97	214.9 ± 83.11
Calcium as calcium Carbonate	36.46 ± 8.64	14.6 ± 15.35	22.83 ± 5.35
Magnesium as Magnesium carbonate	49.27 ± 14.69	39.87 ± 18.62	36.60 ± 8.53
Alkalinity	298.6 ± 101.80	159.4 ± 86.44	218.6 ± 101.80

Analysis of Heavy metals in water

Due to urbanization and industrial processes large quantities of pollutants have continuously been entered into ecosystems. Metals are persistent pollutants that can be biomagnified in the food chains, becoming increasingly dangerous to human and wildlife. This has led to the development of monitoring schemes aimed at directly measuring levels of contaminants in various organisms, and biomonitoring schemes that use indicator species to estimate the levels in other parts of the ecosystem.

Birds, like other organisms, are harmed by heavy metals. For example, metals were affect birds immune system, increase aggressive behaviour, territorial song, and reproductive dysfunction, increased susceptibility to disease and stress and changes in behavioural pattern.

Heavy metals are frequent waste products of industrial and agriculture processes, they enter the food chain via air, water, soil, and biota and their accumulation increases at higher levels of food chain (Burger, 1993). Heavy metals can have harmful effects on development, behaviour and intelligence both in animals and humans (Finkelstein *et al*., 1998). In this study Cd, Cr, Pb, Hg, Mn and Zn were tested for all five feeding sites of *Ardeola grayii*, it was observed that, in all feeding sites Cr, Mn, and Zn was present in equal proportion than Pb, Cd, and Hg, first three Cr, Mn, and Zn not too much harmful than Pb, Cd, and Hg. In this study for all three seasons data were compared by using error bar graph, and Heavy metals present in water for all five sites and three seasons.

Heavy metals present in different study sites

Ardeola grayii prefer to feed near water bodies, to know which feeding area have more metal contamination, error bar graph was made. In this study it was find out cadmium concentration almost similar in all five study sites, (S1- 0.14±0.18, S2- 0.07± 0.01, S3- 0.16 ± 0.01, S4- 0.14 ± 0.01, and S5- 0.21 ± 0.02, respectively). The concentration of chromium was highest in site 3 0.21 ± 0.28, lowest in site 1 0.05 ± 0.04. lead was highest in site 5 20.8 ± 2.5, lowest in site1 0.11 ± 0.04. Manganese was highest in site 5, 0.21 ± 0.04, lowest in site 2, 0.04 ± 0.05. The degree of freedom and test for cadmium was df = 2,42, F = 2.95, and value of p = 0.03, for chromium df = 4,40, F = 2.45, and p = 0.05, for lead df = 4,40, F = 163.88, and p = 0.00, for manganese df = 4,40, F = 2.29, and p = 0.07, and for zinc df = 4, H = 32.32, and p = 0.00. The zinc concentration was highest in site5 0.23 ± 0.02. the error bar graph was given in below figure.

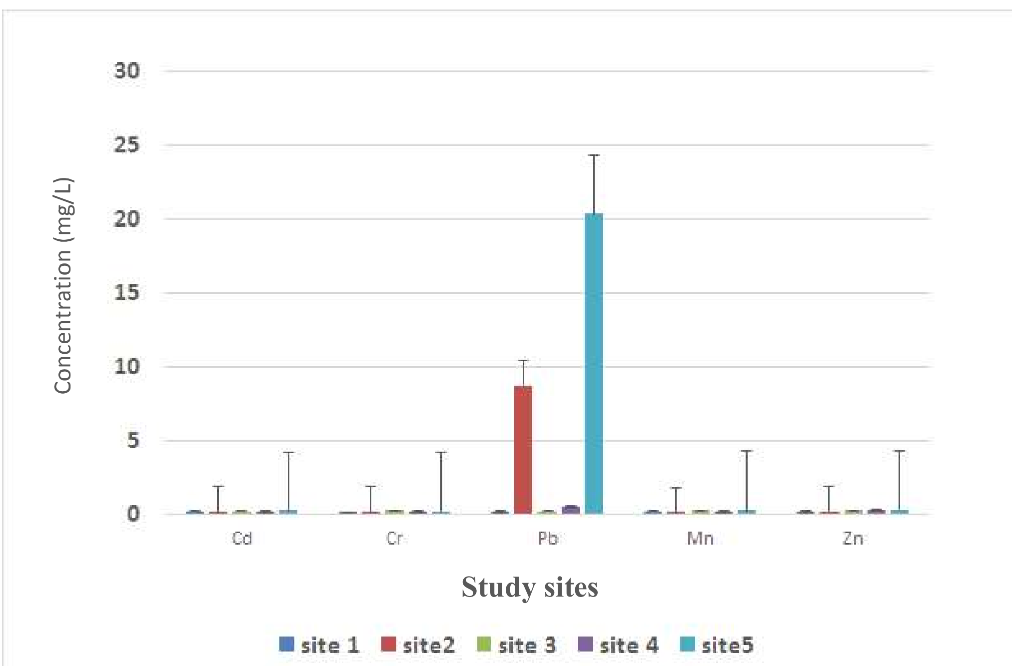

Figure 2.1. Heavy metals concentration in water in different study sites

Heavy metal present in water in different seasons

Heavy metals present in water in different seasons varied for five metals. The concentration of cadmium was present in all study sites almost equal in proportion (Summer 0.17 ± 0.13, Winter 0.12 ± 0.06, and Monsoon 0.14 ± 0.06). the concentration of chromium was also equal in proportion, concentration of lead was highest in monsoon (7.04 ± 9.06), lowest in summer (4.75 ± 6.9), and manganese concentration was highest in monsoon (0.21 ± 0.22), lowest in summer 0.08 ± 0.05. the concentration of zinc was almost equal in proportion (Summer 0.17± 0.03, Winter 0.19 ± 0.04, Monsoon 0.20 ± 0.04). The error bar graph was given below figure 2.2.

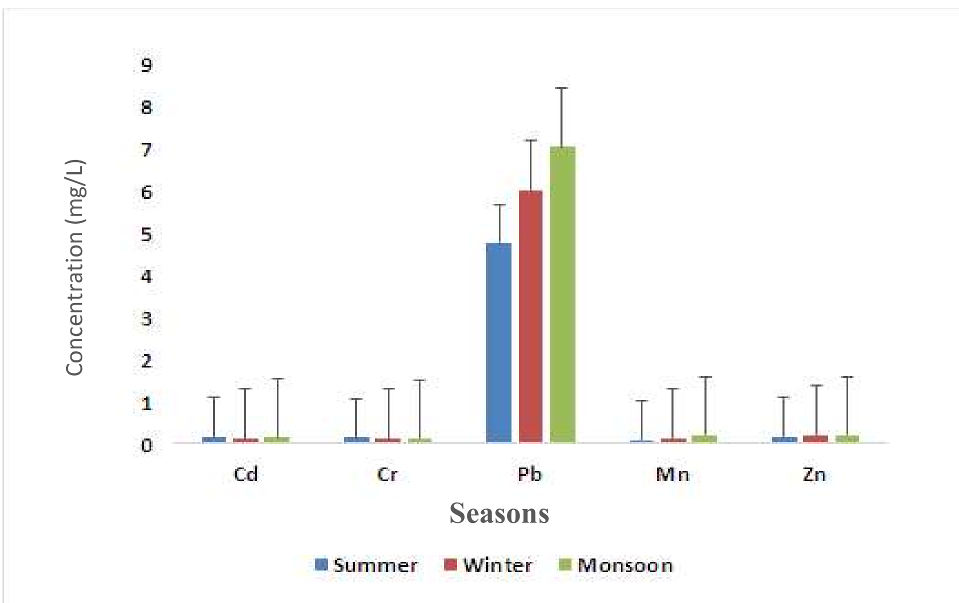

Figure 2.2. Heavy metals concentration in water in different seasons

The values for cadmium statistics test and degree of freedom was df = 2, 42, F = 0.99, and p = 0.37, for chromium df = 2, 42, F = 0.08, and p = 0.92, for lead df = 2, 42, F =

0.28, and p = 0.75, for manganese df = 2, 42, F = 3.06, and p = 0.05, and for zinc df = 2, H = 6.16, and p = 0.04.

Effect of pollutants on the behaviour and population of *Ardeola grayii*

Pollutants present in environment indirectly affect birds, in this study it was observed that in less contaminated habitats *A. grayii* preferred to feed solitary, and waited for longer to capture prey in same habitats, in contaminated habitats it frequently changes habitats (Himangshu, 2017), compelled to share habitats with other species or same species, that leads to fighting among them, in contaminated area fishes, insects, annelids, amphibians not present, so it has to feed a low quality food. Their number in every habitat, site1 to site 5 declined from 2016-2019 in hundread to ones. Pollutants and their effect on *A. grayii* listed in below table 2.6.

Table 2.6. Different pollutants present in feeding habitats and their effect on behaviour of *Ardeola grayii*

Feeding sites	Pollutants	Change in behaviour	Number of *A.grayii*
Site-1	Domestic waste disposal site,	Flee behaviour, changes in flight distance	115-35
Site-2	Misuse of pond as sewage and domestic waste disposal site , water hyacinth	Flee behaviour, aggression, scavenging behavior	95-8
Site-3	Being in proximity to religious complex, people use plastic bags, matchsticks, incense sticks, milk packets, disposable utensils, earthen pots, etc. that are often carried to pond by winds.	Flee behaviour, increase in tolerance level	24-10

Site-4	Dumped garbage in pond	Not much contaminated as other sites, normal behaviour seen	25-17
Site-5	Tannary waste, automobile Wastages	Flee behaviour, scavenging behaviour	20-8

Behaviour is suggested to be a more useful indicator or biomarker than standard assays in laboratory conditions because the harmful effects of pollutants sometimes become only noticeable in natural ecological conditions, such as social stress or infections (Zala and Penn, 2004). In this study it was observed that, *Ardeola grayii* behaviour differed in urban and rural area, because urban areas more polluted than rural area. Site 2 and site 5 was polluted compare to other three sites.

Site 1 was large in size and it inhabitants many waterbirds, it was not much contaminated, but human disturbance occurs there, as a result *Ardeola grayii* seen to frequent change foraging patches.

Anthropogenic activities are some of the major factors in the study area posing significant threat to these wetlands. Water hyacinth has rapidly covered the water surface in Site2 Lake, thereby, reducing the foraging area for open-water birds. These large, unwanted monotypic stands of water hyacinth could reduce the value of the wetland as potential *Ardeola grayii* habitat (Manral and Khudsar, 2013). Site 3 was polluted due to dumping of wastes materials (such as plastics, polythene bags, chips packets), bathing and offering made in the ponds, during mass bathing by local people are influencing the water quality and avifauna.

Site 4 was in rural area less polluted only fleeing behaviour observed there. Site 5 was also in rural area but it was near to tannery factory so water contaminated.

DISCUSSION

The physico-chemical parameters of water function in many way, influences upon biotic component of that ecosystem, thus above certain level of variation is become pollutants for all living organism. DO along with BOD and COD are important parameters in water quality assessment and indirect indicators of organic matter and industrial waste present in a water body. Chloride is excellent parameters to judge the organic pollution in the water. Cation of calcium, magnesium, iron and manganese contribute to the hardness of water (Shrivastava and Patil, 2002). Calcium, one of the most abundant substances of natural water is also added along with sewage and industrial waste.

Permissible limit of water for wildlife and other animals according to CPCB is for pH it should be 6.5-8.5. The present study pH range is between 6.1-8.3, study site 2 and 5 have high pH which indicate water is gradually contaminating but other sites (S1, S3, and S4) are in favourable condition for survival and growth of aquatic organism.

DO along with BOD and COD are important parameters in water quality assessment and indicators of organic matter and industrial waste present in water body. Low levels of DO in the water indicate stress problem for aquatic organisms and in a healthy lotic ecosystem the level of DO is generally close to saturation (7 mg/L) (Mulani *et al*., 1993). The present study indicate site 2 and site 5 having low DO and high level of BOD and COD because in these water bodies too much load of anthropogenic activities.

High values of both BOD and COD indicate high levels of organic pollution in water. Sampath and Sharam (2003) attributed higher levels of BOD (8.2- 35.2 mg/L) in few stretches of Periyar River to sewage pollution. The approximate COD/ BOD ratio for untreated domestic sewage is >2 (Manral and Khudsar, 2013) requiring biological treatment and other treatments if it's more.

Chloride is excellent parameters to judge the organic pollution in the water. Cation of calcium, magnesium, iron and manganese contribute to the hardness of water (Shrivastava and Patil, 2002).

It is important to assess the alkalinity of water bodies to determine the ability of neutralizing the acidic pollution of water from rainfall of waste water. Total alkalinity is measured on the basis of some components such as bicarbonate, carbonate and hydroxide. According to Durrani (1993) withdrawal of CO2 from the bicarbonates for photosynthesis by algae may increase total alkalinity. In this study S-2 and S-3 was having high alkalinity.

Seasonal variation in Physico-chemical parameters of Water in different study sites

The concentration of pH was highest in winter, lowest in summer. The maximum concentration of pH in winter is due to phytoplankton growth, minimum pH in summer is associated with low photosynthesis.

The highest dissolved oxygen value was found in winter, lowest in summer. The highest value of dissolved oxygen may be due to low temperature, while lowest value in summer is due to highest temperature and decomposition of organic matter.

A high Biological oxygen demand value indicate the presence of microorganisms, which show a high level of pollution. Biological oxygen demand was higher in summer followed by winter and monsoon.

Chemical oxygen demand was also higher in summer followed by winter and monsoon. High COD may cause oxygen depletion on account of decomposition of microbes to a level detrimental to aquatic life.

Total alkalinity was maximum in summer and minimum in winter, the high value of alkalinity is due to presence of nutrients in water and presence of excess of free CO_2. The alkalinity is lower due to dilution of water.

High chloride content in the water during winter may be due to low volume of water in the reservoir. Low chloride concentration in summer may be due to dilution of water.

Aquatic heavy metal pollution usually represents high level of Hg, Cr, Cd, Pb, Mn, Zn in water system. Some heavy metals are essential for life process, but some metals such as Cd, Cr, Pb, and Hg are harmful if it will go to beyond a certain limit.

Cadmium is harmful for human, animals, birds, and aquatic organism, it causes hypertension, arteriosclerosis, cancer. According WHO and EPA (2002), acceptable concentration is 3-5µg/L, but according to CPCB (2008) there is no relaxation for it. In this study concentration of Cd was less than other metals. Concentration of cadmium was almost equal in three seasons and all study sites.

Lead and mercury causes autoimmunity disease, resulted malfunction in many organ systems. In this study site 2 and 5 have maximum concentration of both metals that shows these sites become lethal for survival of any organism. In monsoon season concentration of lead was highest.

Chromium is trace metal for birds, but in higher concentration causes some disease. Manganese concentration in water was higher in all five study sites, it was accumulated in water due to release of fertilizer, varnishes, fungicides and livestock feeding supplement in water body. It also bio accumulated in lower organism such as algae, mollusc, and some fishes. The concentration of chromium was almost equal in all three season.

Zinc is an essential growth element for plants and animals but at elevated levels it is toxic to some species of aquatic life. In this study all five study sites have higher concentration of it, it was due to all study sites near to automobile agency, so effluent of workshops entered in water bodies, that causes increase concentration of it. In this study it was observed that in monsoon season concentration of heavy metals is higher compared to other seasons.

Ardeola grayii is sit and wait predator, but in this study it was observed that scavenging behaviour also observed in some feeding sites. This birds show commensalism with human and having wide niche occupancy, such behaviour is important for urban adapted species. *A. grayii* preferred to feed near agriculture fields, ponds, lake, and other water bodies, but due to shrinkage of water bodies, it was seen near garbage dumps in towns, waste water canal. In urban area it has to forage at the closest site which provides high quality resources but they forage at local sites which provide lower quality of food if there are minimal energetic expenses from travelling or completion. *Ardeola grayii* is highly susceptible to continuous anthropogenic pressures in the form of washing clothes, cattle bathing, cattle grazing, and entry of domestic sewage, hunting, fishing, and expansion of crops lands. Pollution present in our ecosystem is one of the most horrible disasters. Every single activities of human society have produced negative impact on all living organism. The cause of water pollutants is domestic sewage, detergents, pesticides, chemicals, dead materials and industrial effluents through a variety of processes. A healthy ecosystems can save from harm to the organisms existing within them, including humans, necessitates not only ecological planning and management, but also knowledge of how stressors vary in the atmosphere (Burger *et al*., 2004). More and more it is essential to appreciate the outcome and effect of pollutants to evaluate the health of ecosystems and to bring early warning of alterations in the environment that might specify undesirable effects (Burger, 2002). Wetland bird's populations and behaviour may provide as sentinel species for natural and anthropogenic pollution problems in the surroundings.

In this study it was mainly focused on there any impact of water quality on *Ardeola grayii*, it was observed that, it is fish loving bird, and fishes are found in lake, pond, agriculture field, and small ditches, but if there was not life supporting requirement of fishes in water so there was very few or almost fishes absent in that feeding sites as a result *A. grayii* had to change that site for feeding or shifted foraging behaviour. In urban area due to lack of water bodies or if available choked by garbage and get contaminated so there was no life support for aquatic organism as a result *Ardeola grayii* shifting their dependency on water bodies to garbage or dumped area, where it can get insects, worm, and bugs. But in rural area there was water bodies available so *Ardeola grayii* mostly observed near lake, pond or agriculture field for foraging, there was less disturbance, prey easily available, no polluted water and their number was maximum than urban areas.

In this study it is observed that, water quality affect behaviour of *Ardeola grayii* and their population in any feeding habitats, we should know the importance of water bodies and wading birds for ecosystem and food chain, we should try not to dumped garbage in water bodies, it importance not only for wading birds but also for fishes and other invertebrates which live in water bodies and all those are play important role in maintaining a food chain.

Shrinkage of water surface, decrease in salinity and fishery resources, introduction of invasive fresh water aquatic weeds is the greatest threats to the lake. An overall loss of biodiversity with decline in productivity adversely affecting the livelihood of the community.

CONCLUSION

Ardeola grayii is mostly depend on water body for their life activities, this studies data suggests that, gradually shrinkage of water body and deterioration of water quality increasing so in near future aquatic birds will come under threatened condition. In this study the feeding habitat of *A. grayii* exhibits low DO, high BOD and COD, total hardness and higher level of concentration of metal level.

In this study it was discuss that, variation in physico-chemical parameters of water in studied water bodies was gradually deviating from permissible values as recommended by CPCB. It was also noticed in this study, *Ardeola grayii* adopting different behaviour and changing habitats, but habitat change alters the moment of migration, breeding success, and distribution of population, which was not good indication for this bird. Hence, it is necessary to restore the ecological properties in order to sustain these birds with in the area.

Chapter-III

The accumulation of heavy metals on feathers, feces and diets of *Ardeola grayii*

CHAPTER-III The accumulation of heavy metals on feathers, feces, and diets of *Ardeola grayii*

INTRODUCTION

Heavy metals are not always harmful for living organism it plays vital role in lives of living species (Dixit *et al*., 2015). In nature heavy metals are present in trace levels in the structure of all living creatures, if these levels are exceeded shows noxious effect in the organism (Zolfaghari *et al*., 2007; Manjula *et al*., 2015). Metals like mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), cobalt (Co), and zinc (Zn) are highly toxic for all components of the ecosystem (Burger and Gochfeld, 1997; Gerbersmann *et al*., 1997; Lee *et al*., 2006) and it also shows severe impact on the environment and stability of ecosystem (Guven *et al*., 1999). Some metals are categorized into essential and non-essential metals. Lead (Pb) is non-essential heavy metals for organisms and sources of lead are from burning of fossil fuels, road traffic, and industries. It is indicator of metal pollution caused by anthropogenic sources (Metcheva *et al*., 2006), including sewage disposal, fuel combustion, waste incineration, paints, vehicles, polishing, leather industry and oil spills (Jerez *et al*., 2011; Markowski, 2013). It is mostly present in calcareous tissues and in feathers of birds. Cadmium is also a non-essential element, present in the environment as a result of anthropogenic activities, with detrimental effects for birds on egg formation, testicular damage, oviduct malfunctioning and kidney damage (Malik and Zeb, 2009). When concentration of Cd is higher it will interfere with replacement of essential elements at the active sites of biologically important biomolecules, thus indirectly impact on nutrients deficiencies (Furness, 1997).

Lead and cadmium both are harmful for avian species (Scheuhammer, 1987) if it will intake through diet, directly influence the endocrine system (Stoica *et al*., 2000; Martin *et al*., 2003), kidneys (Nordberg, 1971), reproduction (Buerger *et al*., 1986), behavioral response (Hui, 2002), molting, migration (Honda *et al*., 1986), enzymes involved in hemoglobin formation, and growth rates (Eisler, 1988). Chromium is include in non-essential elements for living organism and its impact on reproductive process of different avian species (Malik and Zeb, 2009). Some of the study area is near to leather industry, and the high concentrations of Cr can be related to the extensive use in tanning process of Cr salts, it was disposed in environment as effluent and sludge and finally it entered in ecosystem via bioaccumulation from lower trophic level to higher trophic level through food chain (Khan *et al*., 2013). Mn is essential elements, it involved in several biochemical reactions in animal as well as plants. Mn is generally present in igneous rocks, but due to uncontrolled traffic pollution, disposal of untreated wastages in environment, contributing Mn burdens in the environment (Zayed *et al*., 1999). Zn is also comes under essential elements it involved in many enzymatic systems but sources of zinc used in alloying, dyeing, and manufacturing electric goods, insecticides, and cosmetics (Irwin, 1997), and causes reduced species diversity and abundance, as well as fainting, nausea, and stomach disorders (Perez-Lopez *et al*., 2008).

Birds plays important role in ecosystem, they are having diverse and evolutionary successful species and occur in large number in tropics. They are indicator of environmental problems, and indication of healthy ecosystem. *A. grayii* forage on various habitats, where they prey on Insects, Annelida, Amphibians, Mollusc, and Fishes. In food web *A .grayii* is high on trophic level, they are useful as a bio indicator species for environmental pollution, especially aquatic environments. In this study non-invasive samples of birds such as feathers, feces, and diet is used for metal analysis. By observing concentration of metals in specific parts of sample and determine which sample is a mostly accumulated metal and it also determine which feeding sites are more contaminated. However, the feathers, feces, and diet (Dauwe *et al*., 2004; Ek *et al*., 2004) are collected from breeding and feeding areas of *Ardeola grayii*. Herons are located in upper level of food web provides information on the status and condition of the related ecosystem (Burger, 1993). In such a way, it is not only providing information on heavy metal concentration in *Ardeola grayii* but also heavy metal concentrations in other living organism in lower trophic of the food web (Battaglia *et al*., 2005).

Herons comes contact with these metals through soil, air, and water, metals absorbed through the intestinal tract, circulate through the body, and deposited in different body organs, and excreted directly or sequestered in feathers (Furness *et al*., 1986). Female birds excrete some metals in their eggs and eggshells (Fasola *et al*., 1998). Thus monitoring environmental contaminants such as heavy metals in any area, birds are used as good indicator of not only health of their species but also health of ecosystem where they live.

MATERIALS AND METHODS

Study area

The study was carried out in different areas of Lucknow city (upto 50 km) from September 2016 to February 2019, in three season, Summer, Winter, and Monsoon, studied site was divided into 5; site 1 (2644’16” N, 8057’18” E), site2 (2645’31’’N, 8056’32’’E), site 3 (2633’21’’N, 8030’38’’E), site 4 (271’20” N, 8050'7''E) and site5 (2645'34''N, 8051'21''E), all these sites were used by *Ardeola grayii* for breeding or feeding purposes.

Sample collection and preparation

Feathers

Feather sample were collected in different areas of Lucknow in three season, site 1 (Kalli paschim), site 2 (Mawaiya), site 3 (Behasa), site 4 (Deva), and site 5 (Unnao). Feathers were collected from breeding and feeding habitats of *Ardeola grayii*, during sample collection we did not disturb the birds, white sheet spread behind nesting trees and fallen feathers collected from feeding sites.

Concentrations of six metals, namely, Cd, Cr, Pb, Hg, Mn, and Zn, were analysed, every month three samples from each sites for three season. Firstly, a minimum 2g of feather samples were washed 3 times with tap water, rinsed with distilled water, and then washed in acetone (Battaglia *et al*., 2003) to get rid of the external pollutants (Goede and Bruin, 1984). One gram of feather sample was digested in a microwave digestion system using 10ml concentrated nitric acid HNO_3 acid for 10 min followed by 1 ml perchloric acid $HClO_4$ for 5 min and 7 ml of hydrogen peroxide H_2O_2 for 10 min at 220 W power (Jayakumar and Muralidharan, 2011). Solutions were made up to 50 ml with deionized water. Duplicate samples were prepared using the same procedure. The digestion was carried out by using a hot plate with a magnetic stirrer, initially at low temperature and then at higher temperature. The digested samples were filtered, stored in amber vials, and subjected for heavy metal residual analysis using atomic absorption spectroscopy. Prior to feather sample analysis, the instrument was calibrated using standard solutions of each metal obtained from Fisher Scientific Company.

Feces and food

Food and feces of *Ardeola grayii* were collected by spreading white transparent sheet beneath nesting and roosting sites and samples collected in sterile plastic bags with the help of spatula. The samples were collected from five feeding and nesting sites of *Ardeola grayii*. Samples were pooled 0.5 gram excreta were taken with and (3:1) ratio of concentration 5ml perchloric acid and conc. Nitric acid was added. A blank was also prepared without samples. Sample were run separately for feces and food sample, some food sample could not digest easily, so timing of digestion differed. The samples were then digested in microwave at 121^{o}C for 50 minutes. The final volume was made to 25ml by adding distilled water and then the solutions were filtered. The digested samples were analysed for the assessment of heavy metals like cadmium (Cd), chromium (Cr), lead (Pb), manganese (Mn), Zinc (Zn), and mercury (Hg) by Atomic absorption spectroscopy, the reading taken on AAS were converted into parts per million (ppm = µg/g). Procedure was followed Muralidharan *et al*., (2004) and Aggarwal (2002).

Calculation

Calculation for all three sample (feathers, feces, and food) done by following formula

Metal concentration= Dilution factor divide Weight of sample

Dilution factor=10

Dry weight of the sample= 0.5 gm

Metal analysis was done by using atomic absorption spectrophotometer at 217 nm for lead, 228.9 nm for cadmium, 213.9 nm for zinc and 357.9 nm for chromium, 253.7nm for mercury and 279.5nm for manganese. Results are presented in µg/g (ppm). Calibration curves were prepared separately for each metal and for every sample (feathers, feces, and food) using different concentrations (0.5 ppm, 1 ppm, 2 ppm, 5 ppm and 10 ppm) of standard solutions.

STATISTICAL ANALYSIS

SPSS (version 21.0) was used for the statistical analysis. Before using any statistical test, checked normality according to distribution of data, test was used for further analysis. In all three season concentration of metals for all five sites S1 – S5 statistically significant ($p < 0.05$), therefore, ANOVA and Kruskal–Wallis H test was used to determine the differences in metal concentration in all three season and for five study sites. Standard error bar graph was made with the help Microsoft excel, mean and SD value was also calculated.

RESULTS

Metal analysis through feather, feces and food of *Ardeola grayii* is non-invasive tool and there is no harm to birds. The concentration of metals presents in all these sample (feather, feces and food) are analyzed for all three season and in five study sites.

Metal present in feather

Metal analyzed in feather of *Ardeola grayii* in five study sites varied significantly. Cadmium, chromium and lead differ significantly ($p < 0.05$), but for manganese and zinc level did not varied significantly ($p > 0.05$). The distribution of data was analyzed by Shapiro-Wilk W test. As the distribution of data was normal one-way ANOVA, if data are not normal Kruskal - Wallis test is used to explored the variation in concentration of metals present in different sites.

To see variation in metals concentration in feathers of *Ardeola grayii* in different study site, one-way ANOVA and Kruskal - Wallis test was done.

Table3.1. Concentration of heavy metals in the feathers of *Ardeola grayii* in different feeding sites, the values are given in mean ± SD

Metals (μg/g)	Site1 (Mean±SD)	Site2 (Mean±SD)	Site3 (Mean±SD)	Site4 (Mean±SD)	Site5 (Mean±SD)	F/H*	P
Cd	1.57±0.66	1.84±0.62	1.40±0.44	1.73±0.33	1.88±0.30	1.53	0.21
Cr	5.75±2.07	4.62±2.25	6.47±2.61	5.39±2.59	4.52±0.78	1.26	0.30
Pb	1.59±068	2.78±3.58	1.12±0.33	1.27±0.56	2.25±0.24	1.59	0.19
Mn	13.58±3.82	18.18±6.61	14.33±5.65	17.22±6.79	19.05±8.10	4.38*	0.35
Zn	48.12±5.50	50.09±4.66	46.95±4.11	34.22±3.32	47.88±7.39	21.60*	0.00

(*Kruskal-Wallis H test)

When we campare site wise concentration variation in five sites only zinc was varied significantly in all five study sites (Table 3.1.).

In all three seasons concentration of metals present in feather varied significantly for Cd, Cr, and Pb, but for Mn and Zn did not varied significantly. The degree of freedom for in all study sites was 4, 40 (df = 4, 40) for F test and 4 (df = 4) for H test.

Table 3.2. Concentration of heavy metals in the feathers of *Ardeola grayii* in different seasons, the values are given in mean ± SD

Heavy metals	Summer (Mean±SD)	Winter (Mean±SD)	Monsoon (Mean±SD)	F/H*	P
Cd	1.19±0.44	1.79±0.37	2.05 ± 0.29	18.17	0.00
Cr	4.80±2.24	5.46±2.24	5.71 ± 2.33	0.60	0.55
Pb	1.27±0.72	1.74 ±0.48	2.02 ± 0.40	12.65	0.00
Mn	9.39 ±2.63	17.21±2.91	22.61 ± 4.68	14.92	0.00
Zn	40.55 ±6.24	46.07±6.80	52.86 ± 3.37	31.47*	0.00

(*Kruskal-Wallis H test)

Metals present in three seasons varied for four metals, cadmium, lead, manganese, and zinc concentration were differed in three seasons, but concentration of chromium in three season did not varied. The value for degree of freedom for three seasons were 2, 39 (df = 2.39) for F test and 2 (df = 2) for H test.

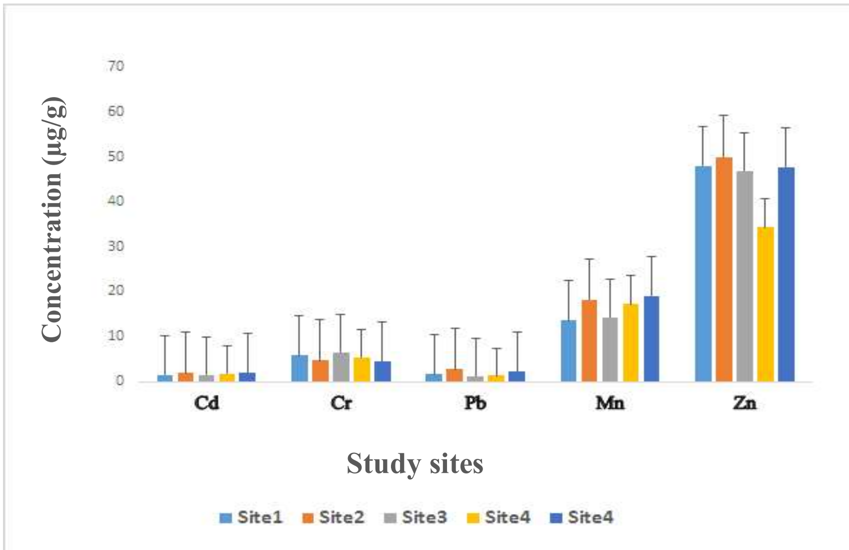

Figure 3.1. Concentration of heavy metals in feathers of *Ardeola grayii* in different study sites

Heavy metal concentrations (Cd, Cr, Pb, Mn, and Zn) in feathers of *Ardeola grayii* in five feeding sites comparison are shown by error bar graph illustrations with mean and standard error (Fig. 3.1). Heavy metal concentrations are statistically significantly between concentration and study sites ($p < 0.05$). Metal concentration of *Ardeola grayii's* feathers are decreasing order: Zn > Mn > Cr > Cd = Pb was found in five sites. The average concentration of Cd recorded for sites S1 to S5 are; (1.51± 0.6), (1.84 ± 0.62), (1.40 ± 0.44), (1.73 ± 0.33), (1.88 ± 0.30) respectively. The concentration of chromium is 5.75 ± 2.07, 4.62 ± 2.25, 6.47 ±2.61, 5.39 ± 2.59, 4.52 ± 0.78, for study sites S1 to S5 respectively. The Pb concentration in feathers of *Ardeola grayii* was found ranging 1.59 ± 0.68, 2.78 ± 3.58, 1.12 ± 0.33, 1.27 ± 0.56, 2.25 ± 0.24, in the five sites S1 to S5, respectively. Whereas, the concentration of manganese (Mn) are, 15.53 ± 1.40, 18.18 ± 6.61, 14.33 ± 5.65, 17.22 ± 6.79, 19.05 ± 8.10 in five sites S1to S5 respectively. While the concentration of zinc are ranging, 48.12 ± 5.50, 50.09 ± 4.66, 46.95 ± 4.11, 34.22 ± 3.32, 47.88 ± 7.39, for five sites S1 to S5, respectively.

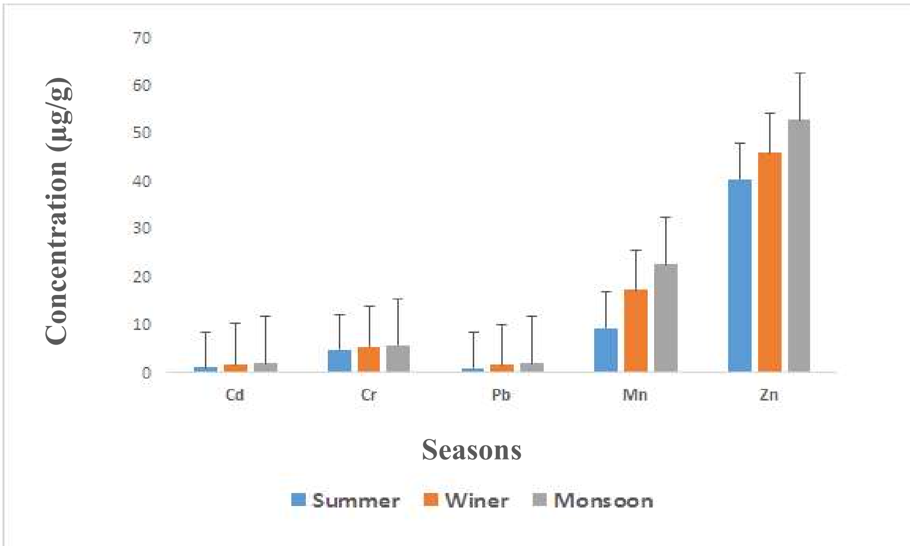

Figure 3.2. Concentration of heavy metals in feathers of *Ardeola grayii* in different seasons

Heavy metals present in feathers of *Ardeola grayii* varied in concentration in different seasons. Cadmium concentration in summer is lower (1.19 ± 0.44), compare to winter (1.79 ± 0.37) and monsoon (2.05 ± 0.29). chromium was higher in monsoon (5.71 ± 2.33) and winter (5.46 ± 2.24), lower in summer (4.80 ± 2.24). lead concentration in summer was 1.02 ± 0.66, winter 1.74 ± 0.48, and in monsoon 2.02 ± 0.40. manganese concentration in summer was 9.39 ± 2.63, winter 17.21 ± 2.91, and higher in monsoon 22.61 ± 4.68. zinc concentration was highest than other four metals in summer, winter, and monsoon, 40.55 ± 6.24, 46.07 ± 6.80, and 52.86 ± 3.37 respectively.

Concentration of heavy metals in feces of *Ardeola grayii*

In this study feces of *Ardeola grayii* was used for analysis of heavy metals present in different feeding/nesting sites. Variation in metal concentration in different seasons and sites were shown by standard error bar graph and Table 3.3. The degree of freedom for five metals in each seasons was 2,42 (df = 2. 42), in Table 3.3 only one-way ANOVA test was applied due to normally distributed data.

Table 3.3. Variation in concentration of heavy metals in feces of *Ardeola grayii* in seasons

Heavy Metals	Summer (Mean±SD)	Winter (Mean±SD)	Monsoon (Mean±SD)	F/H	P
Cd	0.36±0.24	0.47 ± 0.26	0.49 ± 0.32	0.98	0.38
Cr	7.99 ±3.85	9.08 ±4.19	8.90 ± 3.50	0.34	0.71
Pb	15.40 ±17.75	7.01 ±3.21	9.21 ± 4.56	2.45	0.09
Mn	33.36 ±17.12	40.00 ± 13.56	43.21 ±12.80	1.77	0.18
Zn	31.30 ± 12.91	38.14 ± 9.22	40.53 ± 9.78	2.96	0.06

The concentration of cadmium was recorded ranges from 0.10-0.69 µg/g which was less than normal ranges recommended by CPCB (0.02-1.5ppm), minimum concentration was found in summer (0.36 µg/g) and maximum in monsoon (0.49 µg/g). The concentration of chromium was recorded highest in winter (9.08 µg/g) and lowest in summer (8.0 µg/g), lead was highest in summer (33.3 µg/g) lowest in winter (7.01 µg/g), manganese was highest in monsoon (43.21 µg/g) and lowest in summer (33.3 µg/g), and zinc was highest in monsoon (40.5 µg/g) and lowest in summer (31.30 µg/g).

Table 3.4. Variation in concentration of heavy metals in the feces of *Ardeola grayii* in different study, the values are given in mean ± SD

Metals (µg/g)	Site1 (Mean±SD)	Site2 (Mean±SD)	Site3 (Mean±SD)	Site4 (Mean±SD)	Site5 (Mean±SD)	F/H*	P
Cd	0.44±0.10	0.77±0.08	0.16±0.04	0.15±0.06	0.67±0.20	54.39	0.00
Cr	5.04±0.82	12.07±2.13	7.28 ±0.80	5.34±1.53	13.56±1.39	68.07	0.00
Pb	5.74±1.29	12.45±2.16	4.71 ±0.53	4.99 ±0.69	9.49±3.89	23.00	0.00
Mn	28.85±3.86	56.10±4.51	29.84±9.98	32.05±3.77	53.90 ±5.17	47.47	0.00
Zn	30.87±3.28	50.92 ±3.85	31.60 ±2.42	31.11±2.84	46.65 ±6.13	53.50	0.00

(*Kruskal-Wallis H test)

The cadmium concentration was highest in site2 (0.77 µg/g) lowest in site3 (0.16 µg/g), chromium concentration was highest in site5 (13.56 µg/g) lowest in site1 (5.04 µg/g), lead was highest in site2 (12.45 µg/g) lowest in site3 (4.71 µg/g), manganese was recorded highest in site2 (56.10 µg/g), lowest in site1 (28.85 µg/g), and zinc was highest in site2 (50.9µg/g) lowest in site4 (31.11 µg/g). The degree of freedom for each site was 4, 42 (df = 4,42), data was normally distributed so one-way ANOVA test applied and degree of freedom same for five metals.

When error bar graph was draw between concentration of metals and different sites, it clearly shown in which sites metals was present in maximum concentration.

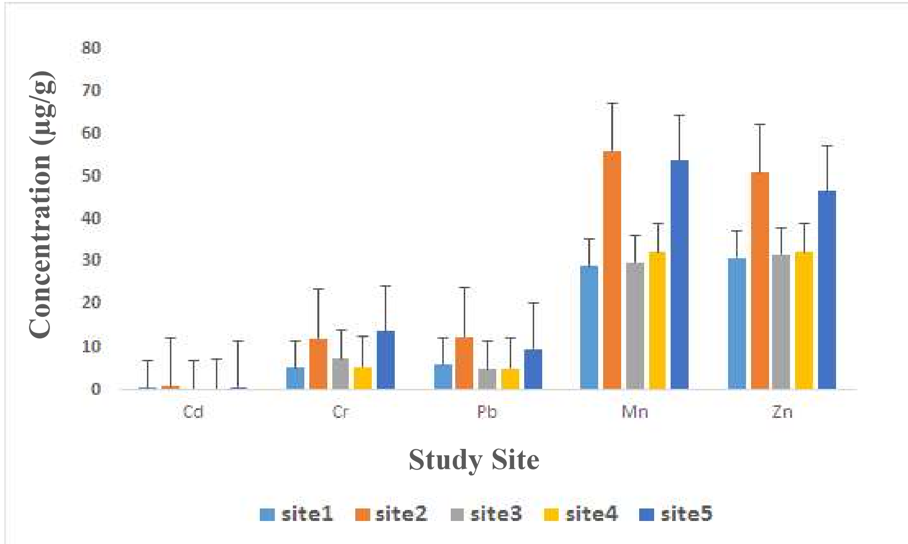

Figure 3.3. Concentration of heavy metals in feces of *Ardeola grayii* in different study sites

When error bar graph was draw between concentration of metals and seasons, it showed that in which seasons maximum concentration of metal present in feces of *Ardeola grayii*.

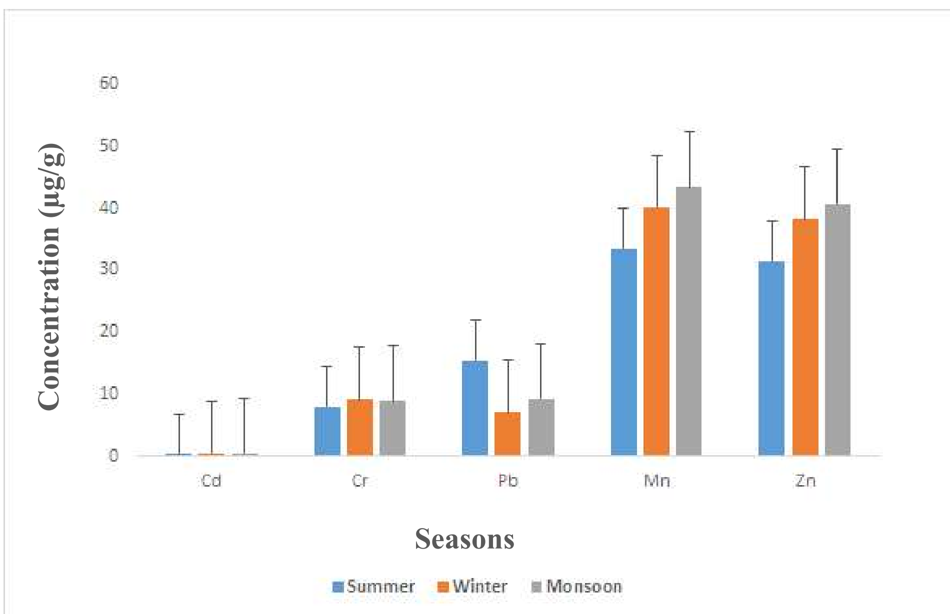

Figure 3.4. Concentration of heavy metals in feces of *Ardeola grayii* in different seasons

Concentration of heavy metals in food of *Ardeola grayii*

Heavy metal analysis through food remnants of *A. grayii* is another non-invasive tool for determination of concentration of heavy metals in different nesting sites. Food remnants were mostly collected below nesting tree of *A. grayii*, the variation of metal concentration was done for three seasons, given in table 3.5-3.6

Table.3.5. Variation in concentration of heavy metals in food of *Ardeola grayii* in different seasons

Heavy Metals	**Summer (Mean±SD)**	**Winter (Mean±SD)**	**Monsoon (Mean±SD)**	**F/H**	**P**
Cd	0.43±0.39	0.62 ± 0.56	0.57 ± 0.34	1.00*	0.60
Cr	1.36 ±0.98	0.88 ±0.37	1.43 ± 0.82	1.33	0.28
Pb	1.09 ±0.49	1.20 ±0.86	1.42 ± 0.83	0.45	0.63
Mn	55.98 ±7.73	57.73 ± 7.30	58.19 ±7.80	0.21	0.81
Zn	41.21 ± 9.66	50.95 ± 12.81	62.62 ±13.21	9.92*	0.00

In different seasons concentration of metals varied, cadmium was present in higher concentration in winter 0.62 µg/g, lowest in summer 0.43µg/g. chromium concentration was highest in monsoon 1.43 µg/g, and lowest in winter 0.88 µg/g. concentration of lead was highest in monsoon 1.42µg/g, lowest in summer 1.09µg/g. manganese was highest in monsoon 58.19µg/g, lowest in summer 55.98µg/g. zinc was highest in monsoon 62.6 µg/g, lowest in summer 41.2 µg/g. The degree of freedom for H test was 2, and for F test 2, 24).

Table 3.6. Concentration of heavy metals in the food of *Ardeola grayii* in different study sites, the values are given in mean ± SD

Metals (µg/g)	Site 1 (Mean±SD)	Site 2 (Mean±SD)	Site3 (Mean±SD)	F/H*	P
Cd	0.45±0.37	0.69±0.57	0.47±0.42	1.08*	0.58
Cr	1.08±0.57	1.68±1.06	0.91±0.39	2.75	0.08
Pb	0.83±0.44	1.90±0.82	0.98±0.37	8.94	0.00
Mn	63.11±10.47	53.79±1.90	55.00 ±2.43	5.81	0.00
Zn	48.14±18.53	51.52±11.78	55.12±13.46	1.38*	0.50

Concentration of cadmium was recorded highest in site 2 0.69µg/g) lowest concentration in Site1 0.45 µg/g. The concentration of chromium was highest in site 2 0.69µg/g, lowest in site 3 0.91 µg/g. concentration of lead was highest in site 2 1.90 µg/g, lowest in site 1 0.83 µg/g. manganese was highest in site1 63.11µg/g lowest in site 2 53.79µg/g. the concentration of zinc was highest in site 3 55.12µg/g, lowest in site 1 48.14µg/g. The degree of freedom for H test was 2, and for F test 2, 24).

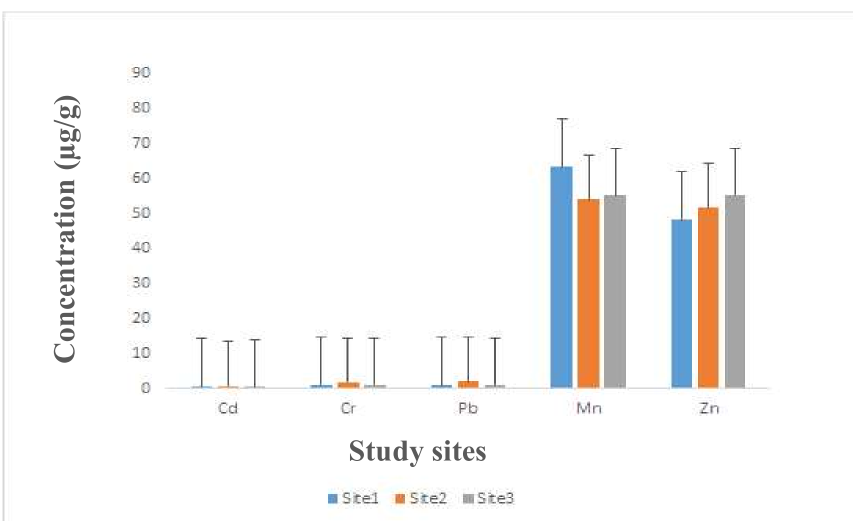

Figure 3.5. Concentration of heavy metals in food of *Ardeola grayii* in different study site

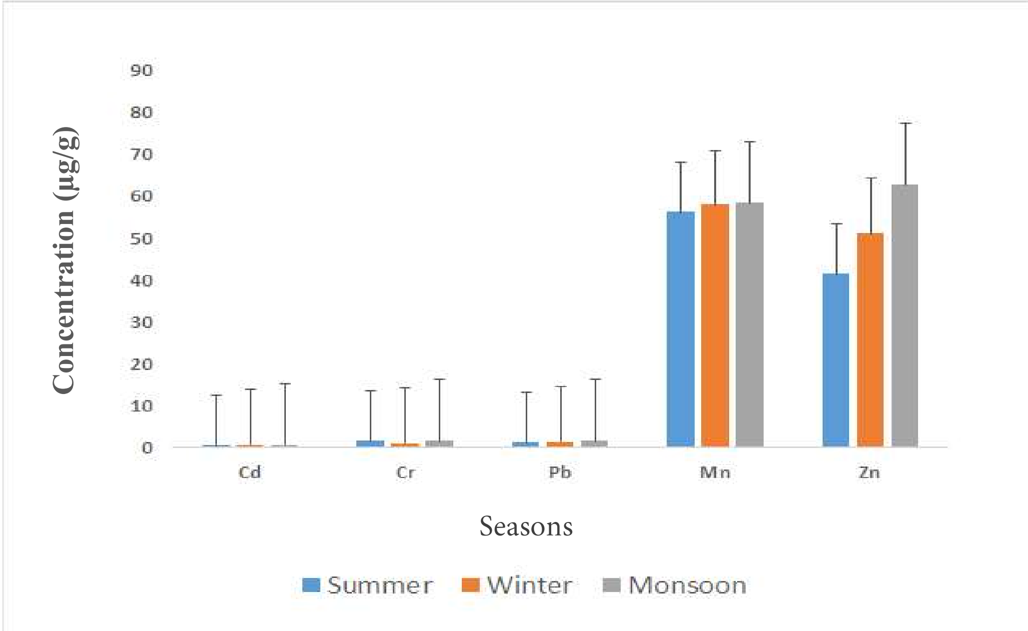

Figure 3.6. Concentration of heavy metals in food of *Ardeola grayii* in different seasons

DISCUSSION

Biggest problem in whole world is environmental pollution which resulted deterioration most of the ecosystem. It is especially true for most developing countries in South Asia, such as Nepal, India, Bangladesh, and Pakistan, where most of the wetlands are about to lost and water pollution increasing due to huge amounts of pollution discharged by urban activities in water bodies (Abdullah *et al*., 2007). About 90% to 95% of all domestic sewage and 75% of all industrial effluent are discharged into surface waters without any treatment (Kumar and Singh, 2002). Lucknow is situated near Gomati river and its rural area have good number of agriculture land and wetlands but day by day due to increasing demand of land, most of agriculture field and wetlands are rapidly converting into building or industrial purposes, these all activities causes loss of flora and fauna. The main sources of environmental pollution are sewage water, domestic garbages, hospital discharges, fertilizers, pesticides, automobiles, industrial effluents, and leather tanning. As a result, soils, air, and water are heavily polluted with various heavy metals like Hg, As, Pb, Zn, Mn, Li, Cd, and Cr (Abdullah *et al*., 2007). Pesticides are major source of lead, cadmium, copper and chromium; also, there are glyphosate-based herbicides that contain metals.

Frantz *et al*., 2012; Kim and Oh, 2012; Manjula *et al*., 2015; Scherer *et al*., 2015; havereported that the avian feathers can be used as bio-monitoring tool of heavy metal pollution. Metals can enter in body of birds through contaminated air, water, soil and food and it accumulate in various tissues. Non-invasive techniques are a crucial way for Heavy metal analysis through feathers, feces, and food sample of birds.

The concentration of metals in feathers, feces and diet of *Ardeola grayii* was highest in monsoon, then winter and summer. The main cause of metal present in water is lowering of pH. In monsoon, due to rainfall and surface run-off, dilution plays important role in lowering pH of water, as a result metals are present in water and finally accumulated in wading birds. Wading birds mostly feed near waterbodies, if metals present in water, it will go to aquatic prey, finally accumulated in birds tissues. Very few work has been done on impact of seasons on metal accumulation in birds body, so we cannot say anything clearly.

In this study some areas are in urban and some in rural, site 1, 2, and 3 in urban, site 4 and 5 in rural. Site 2 and site 5 are severely polluted comparison to other three sites. Previous studied reports concentration of cadmium is lethal for heron if concentration of is greater than 2µg/g in feathers (Burger *et al*., 2004), in this study site 5 cadmium concentration exceed from threshold limits. In this study for all five sites feathers, feces and food remnants of *Ardeola grayii* have variation in concentration of metals, because these all sites have different sources of pollutants.

The non-invasive sampling of feathers indicate that metals adhere to feathers by deposition of chemicals in the atmosphere, from soil, and through preening feathers with oil from the uropygial gland gland. In this study food remnants were collected consists of insects, fishes, earthworms, beetles, grasshoppers, fishes. The metal present in food remnants of *A. grayii* shown that contamination levels of metal present in prey items and surrounding foraging area. The metals present in feces of *A. grayii* has shown that, it reflects the metal concentration in environment and food items captured by birds.

The use of many organic and inorganic fertilizers in agriculture fields increases the bioavailability of metals along with combined effect of pesticides and fungicides (Eijsackers *et al*., 2005). The concentration of these metals was higher in food remnants prey of *A. grayii* metal accumulation in prey of it not only depends on food type but also on feeding habitats and physiology (Burger *et al*., 2004; Burger and Eichhorst 2005; Eeva *et al*., 2009).

In this study, concentration of Mn and Zn in food of *Ardeola grayii* was higher (Khan *et al*., 2013) .

Manjula *et al*., 2015 finds higher concentration of Cr, Cd, Mn and Zn in feathers of different wading and terrestrial birds. In this study, *A. grayii* also accumulate higher concentration of Mn > Zn > Cr > Cd > Pb in their feathers. Dauwe *et al*., 2004, reported metal concentration such as Cd, Pb, and Zn in feces and feathers of nestling songbirds. Because these birds are powerful means to assess the presence of food chain contaminants, feathers are suitable for only some metals not for all.

According to Scheuhammer, 1987 and Burger, 2002 cadmium is toxic to birds and it's harmful impact on egg formation, oviduct malformation, and kidney damage. Greater than 2µg/g of Cd in feather is associated with adverse effects such as kidney toxicity, eggshell thinning, lower food intake, disruption of calcium metabolism and intestinal damage in birds. This study suggested that feathers, feces, and food showed lower concentration of cadmium in summer. Chromium is not an essential element for animals, and it may cause deleterious effect on reproductive health of avian species (Malik and Zeb, 2009). It is observed in feathers of *Ardeola grayii* in five sites exceeding the threshold of 2.8µg/g, (Burger and Gochfeld, 1997) it might be due to anthropogenic source of contamination. Site 2 and site 5 having higher concentration of Cr, while site 5 was near to tannery industry and effluent dumped into water body, and site 2 was contaminated due to anthropogenic sources. Concentration of chromium was higher in feathers and feces lower in food remnants of *A. grayii*. Lead is heaviest non-radioactive element and if ingested, it is poisonous to animals as well as birds. Sources of lead in the environment are pigment in paint, fuel additives, pipes, and glazed ceramic food container etc. it affects birds reproduction, behaviour and hematopoietic and nervous system. If it's concentration greater than 4µg/g in feathers of herons is known to be potentially toxic and directly associated with chronic high level of exposure (Burger and Gochfeld, 1997).

Concentration of lead was higher in feathers of *A. grayii,* this result was supported by Manjula *et al*., 2015; Muralidharan *et al*., 2004; and Burger, 1993. Feces and food remnants also accumulate lead in them but concentration lower than feathers. Mn is an essential micronutrient that plays a vital role in many metabolic and biochemical reactions. Its contamination in food chain occur through aquatic ecosystems, however certain other anthropogenic sources such as urban waste dumps and industrial effluents, vehicular traffic also contribute to it's contamination (Abdullah *et al*., 2005). The high concentration of it in birds can be due to exposure of contaminated dust and ingestion with food. Untreated dumping of traffic waste and other industrial effluents in the foraging area was another possible source of it. It was higher concentration in all three samples (feathers, feces, and food remnants) of *A. grayii*.

Zn is an essential that plays important roles in different metabolic reactions, originate from both natural and anthropogenic sources, uncontrolled waste disposal in or near to water body. In this study in all five sites zinc and manganese both present in higher concentration than other three metals such as (Cd, Cr, and Pb), this is because of all these sites are badly disturbed by anthropogenic sources, and these sites have no any proper disposal of wastage. It was also higher in concentration in feathers, feces and food remnants. These observations throw light on the fact that significant variations in toxic metal levels are present in the environment where this birds live.

CONCLUSION

This study result revealed that feathers, feces and food remnants of *Ardeola grayii* is used as a good bio-monitoring tool for assessment of heavy metals, because it preferred both fishes as well as insects and may visit a variety of habitats with varying degree of contamination. These contaminants not only affects birds but also disturbed a whole ecosystem due to bioaccumulation in lower trophic level to top predator. This study confirmed that accumulation of heavy metals on feathers, feces and food of *Ardeola grayii* provides a reliable non-invasive method of monitoring ecological consequences of the contamination of the environment.

Metals accumulated in body of *Ardeola grayii* impact on their breeding success, as a result its impact on their population. Presently it comes under least concerned but if such situation will continue for long time, it will come under vulnerable list. *Ardeola grayii* a top predator in food chain, ecosystem which was maintained by it will be greatly affected. Hence, we should take steps for proper management of water bodies and proper disposal of wastage so that contamination not reaches to any type of food chain, all organism can survive there.

Summary

Birds are endothermic group of animals, belongs to Class Aves under Kingdom Animalia that include over 10,000 species worldwide, among them, 1340 (13%) bird species were observed from India. Henceforth, India shows a wide range of avifaunal diversity in term of species richness. But as per state of India's bird report 2020, bird species assessed have decline during last few decades. There is decline in bird population in india, as more than 50%. The behavioural ecology of avian fauna deals with all the adaptive circumstances such as, flying, escaping, predation, roosting, feeding, preening, nesting, mating, breeding, parenting, territory defence, communication and migration *etc*. exhibited by the bird throughout the lifetime. For instance, the foraging activity is the way of finding the foodstuff from the natural habitat. It plays a vital function in respect to the capability of animals for their survival and reproduction.

The important behaviour found in birds is the feeding behaviour. Wetland birds are generalized as well as specialized in the selection of food. Generally, the birds are herbivores, frugivorous, grainivorous, insectivorous, carnivorous, omnivorous, predators and some birds are scavengers. The habitat resources such as food, shelter and water are the fundamental requirements of an animal species for their survival and successful reproduction in a particular region.

In the present study it was observed that many factors such as; seasons structure of habitats, depth of water, height of vegetation, types of prey and their size, types of techniques used for capturing prey affects on feeding behaviour, foraging success of *A. grayii.*

This whole study was carried out in Lucknow and its associated rural area, period of study was July 2016-2019 February. This observation was done for three seasons monsoon (July-October), winter (November - February), and summer (March – June). The field visit was conducted five days in week. Study was done in five types of habitats, lake, ponds, agriculture fields, marshy area, and grassland. For observation all activities were recorded by camera and binocular, prey was counted after capturing from sweep net. Some prey which was easily seen by binocular noted in excel sheet. Data were analysed by SPSS (Ver. 21.) and Graph pad prism 5.

Ardeola grayii generally seen near edge of wetlands for feeding and resting purposes, among wetlands most preferred one was lake and pond, because other habitats were seasonally fluctuating, so availability of prey varied there, but in both lake and pond in all seasons prey was available.

A.grayii preferred water depth 10-15cm for capturing prey, less vegetation height, preferred size of prey were, for fishes 1-7cm, insects 0.5-5cm, annelids 3-7cm, amphibians 3-5cm, and mollusc 3-8cm.

Prey was abundant in lake, pond and in agriculture field, but their number varied seasonally, in monsoon seasons, most of the habitats had abundant prey but in winter and extreme summer few prey was available for *A.grayii*.

The foraging success was highest in pond (33.60 ± 29.30) followed by lake (31.87 ± 30.85), Agriculture field (12.07 ± 6.52), marshy area (11.13 ± 11.61), and grassland (9.73 ± 6.49).

In this study foraging success was more with fishes and insects in three seasons and five habitats, increasing order for success was Insects > Fishes > Amphibians> Annelids > Mollusc.

Feeding behaviour and the technique in which they utilize their feeding ground represents the fundamental ecology of that bird's species. Feeding habitat and feeding technique are, therefore, significant factors engaged in resource separation and can be used to understand feeding guilds of the birds. Indian Pond Heron, *Ardeola grayii* feeds solitary as well as in flocks. This large wading bird captures the prey by employing stand and wait strategy, very cautiously capturing the prey by walking slowly towards the prey or by standing still, waiting for the surprise attack, and occasionally hunting the prey when in flight.

Water pollution is becoming a big problem for biodiversity. The main source of water pollution is industrial waste, domestic waste. Sewage wastage directly flows in water body and acid rain which cause deposition of heavy metals in water bodies. Fresh water is a source for the development of civilizations but due to pollution there is severe threat to natural fresh water reservoir. The impairment of water quality due to introduction of pollutants is a problem faced by most industrial cities around the world. Rapid urbanization and industrialization with improper environmental planning often lead to discharge of industrial and sewage effluents into wetlands. The wetlands have a complex and fragile ecosystem, as they do not have a self-cleaning ability and therefore readily accumulate pollutants.

Heavy metals entering the water body would be adsorbed in sediments, and subsequently might migrate as a result of exchanges between water, sediment, and biota, through biological and chemical process. Heavy metals do not degrade in water but are generally not found in high concentrations, primarily due to deposition in sediments but also because of uptake by aquatic organisms. The aim of the present study was to discuss physico-chemical parameters of water in different feeding ground of *A.grayii*, and heavy metals present in water, and impact on their behaviours.

A. grayii use wetlands for their life activities such as, feeding, breeding, resting, and sheltering purposes. In urban area due to day by day shrinking of water bodies, it was compelled to feed near garbage and dumping sites. Changes in physico- chemical property of water affects not only aquatic organism living in water but also wading birds such as *A.grayii, B. ibis, G. chloropus, F. atra, A. atthis, H. smyrnensis, D. leucophaeus.* In order to analyse physico-chemical and heavy metals in wetlands, water samples were collected in twice a month in replicates in urban and rural area of lucknow from September 2016 to February 2019 in three season, samples were collected in pre-cleaned polyethylene bottles. Nine physico-chemical parameters (BOD, COD, DO, pH, Total hardness, Calcium as calcium carbonate, Magnesium as calcium carbonate, Chloride, Alkalinity) of water were analysed in five feeding and nesting ground, heavy metals (Cadmium, Chromium, Lead, Mercury, Manganese, and Zinc) were also analysed. The samples used for DO and BOD examination were collected directly in dark DO bottles, some drop of manganous sulphate solution were added to fix DO. After collection stored at room temperature. In five habitats pH was ranges between 6.3 – 6.9, too much fluctuation in pH are stressful and can even be lethal to aquatic organisms, which may circulate in a food chain. Levels of pH too high (> 9) or too low (< 5) can kill aquatic life.

Biochemical Oxygen demand determines the amount of oxygen required for biological oxidation of organic matter with the help of microbial activities. In the present study the value of biochemical oxygen demand ranged between 5.06 to 29.03 mg/L.

Chemical oxygen demand determines the amount of oxygen required for chemical oxidation of most organic matter and oxidizable inorganic substances with the help of strong chemical oxidant. High COD indicates the presence of all forms of organic matter, both biodegradable and non-biodegradable, and hence the degree of pollution in waters.

Dissolved oxygen for all five habitats was 4.3 – 7.36 mg/L, DO > 5 mg/L is considered favourable for growth and activity of most aquatic organisms; DO < 3 mg/L is stressful to most aquatic organisms, while DO < 2 mg/L does not support fish life.

The permissible value of alkalinity as recommended by Indian standards is 200 mg/L. Alkalinity in this study was range from 103.03 – 361.7 mg/L, the lake can be categorized as nutrient rich water body and highly productive on the basis of total alkalinity. If any water body have > 200mg/L alkalinity is good for community production, it is also the measure of buffering capacity of water. It is important to assess the alkalinity of water bodies to determine the ability of neutralizing the acidic pollution of water from rainfall of waste water.

In the present study chloride concentration was found ranging between 22.10 to 59.8 mg/L. Site -2 was having more chloride level due to large amount of organic matter, waste of animals dumped in lake.

In this study the total hardness of water range was 137.7 to 311.36 mg/L in study area. Site 2 had highest 311.36 mg/L total hardness in water. As the content of Ca and Mg in water increases, the content of hardness also shoots up. The average value of calcium hardness for the study period was 29.6-55.4 mg/L, whereas the average value for Mg^{2+} recorded was 22.1-60.7 mg/L for the study period. Calcium and magnesium are the dominant cations in an aquatic body.

Heavy metals can have harmful effects on development, behaviour and intelligence both in animals and humans. Concentration of heavy metals in study sites were varied for five metals Cd, Cr, Pb, Hg, Mn, and Zn. the study period , concentration of lead was maximum in S5 > S2 > S4, cadmium concentration was almost equal in all five study sites, chromium was maximum in site 3, manganese was maximum in site 4, and concentration of zinc was maximum in site 5.When we compare seasons wise concentration of heavy metals for all five study sites, the concentration of cadmium was maximum in summer > monsoon > winter, concentration of chromium was almost equal in three season, lead was maximum in monsoon > winter > summer, manganese and zinc was maximum in monsoon.

The presence of pollutant in feeding habitats of *A. grayii* impact on their feeding techniques used for capturing prey, and also it causes they could not get enough food for their survival, but we know that herons having good adaptation capability to any conditions, so they survived because they adapted or changed their behaviour. Scavenging, fleeing, sudden attack on their own species for food, and aggression, observed in some *A. garyii*. It showed that, they are changing their behaviour for their survival, but these things will happen to some extent, not for always, at last their population will perish, so this is alarming bell for us to do some conservation management of wetlands, so that birds or other aquatic organism survived and maintain their ecosystem, because every predator top to bottom or bottom to top affect each other.

Due to the feeding habitats of aquatic birds near to water resources such as wetlands, canals, dams, etc. they are much more sensitive to water qualities including heavy metals accumulation. The assesment of heavy metals in aquatic birds more feasible. As aquatic birds are constantly maintained habitation around the water resources. They are also natural drivers for several services among them monitoring of ecosystem system condition as bioindicator are well recognized. The aquatic birds are still successfully maintained their population because of food resources such as river, lake, wetlands, cropping and non-cropping fields, etc. so that identification and quantification of heavy metals are significantly important for our living system. Heavy metals have been shown to accumulate in kidney, liver, blood, feathers, eggs and bones. In this study non-invasive methods used for assessment of heavy metals through feathers, feces, and food remnants of *A. grayii*.

Feathers provide thermal insulation and protect against mechanical injuries. Furthermore, they ensure an appropriate aerodynamic surface for flying. Feathers are made of proteins rich in sulphur-containing amino acids, which is why they accumulate heavy metals. Contaminants pass into feathers only during their formation. Among all the possible methods through which heavy metals assessments can be use, feathers, are non-destructive, least cost and easily available components for the assessment of heavy metals. Feathers are a good accumulator and indicator of heavy metals.

Feces of *A. garyii* also a good tool for investigating heavy metals in their feeding and resting habitats, Feces were collected in different areas. Birds ingested heavy metals mainly due to food and water ingestion. Naturally, aquatic birds prefer to feed at margin or inside the water bodies. The ingestion of different food stubs and their size significantly differ in contamination level of heavy metals among juvenile and adults. In this study food remnants were collected from nesting sites of *A. grayii*.

The overall study showed that, feeding sites of *A. grayii* site 2 and site 5 was more contaminated than other three sites. all five metals Cd, Cr, Pb, Mn, Zn, were present in higher concentration level in these two sites, Mercury (Hg) was present in food remnants of *A. grayii* in feeding site 2, in study period 2016-2017 concentration of Hg was 0.044 ± 0.07, in 2017-2018, 0.04 ± 0.04, and in 2018-2019, 0.15 ± 0.05 present. In sample of feces Hg was also present in site 2 and 5. So it was concluded that feces and food remnant accumulate harmful metals such as mercury than feathers. The feeding and nesting sites 2 and 5 of *A. grayii* was more disturbed by pollutants, because, site 2 was near to hospital and densely populated area, most of the domestic and hospital wastages discharge directly in lake, so from 2016 to 2019 population of *A. grayii* and other wading birds declining, some were fly away permanently from that location, but some birds were still feeding and resting there, as a result they are accumulating contamination in their body. Site 5 was near to tannery, automobile workshop, and some local products making industry, that were discharge their wastages in lake and pond, as a result wading birds and other organism which depend on that lake, accumulating pollutants directly or indirectly in their body.

These birds preferred to stay near water bodies such as lake, river, canal, pond, marshy area, agriculture field. But we are disturbing these habitats by our selfishness nature, as a result birds either terrestrial or water birds are declining. All living organism play an important role to balance ecosystem, and these are interconnected to each other directly or indirectly. By disturbing of any one member of ecosystem, the whole system may be affected and resulted one species will be dominated. So here are some recommendations for the conservation of habitats of *Ardeola grayii* so that it will survive in its natural condition.

Lake, river, canal, and pond should be maintained, so that these birds get food and other aquatic organism such as fishes survived there.

There should be a rule for not using these water bodies for construction purposes, because in this study some lake and pond converted into buildings, as a result these birds are restricted to feed near garbage or contaminated area.

Discharged effluent from houses, workshops, factories, and hospitals should be treated properly, and it should be restricted up to a certain limit. Thrive of *Eicchornia crassipes* in lake or river should be stopped, because it decreases the level of dissolved oxygen in water. Heavy metals are serious pollutants in the aquatic environment because of their persistence and tendency to concentrate in aquatic ecosystem, by knowing their source it should not discharge in aquatic body, so that it will be stopped to accumulate in body of any

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HEAVY METAL ANALYSIS THROUGH FEATHERS OF INDIAN POND HERON (*ARDEOLA GRAYII*)

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ABSTRACT : The aim of this study is to determine the heavy metal concentration by using feathers of *Ardeola grayii* from different area of Lucknow. The metals analysed are: cadmium, chromium, lead, mercury, manganese and zinc from different sites in Lucknow during September 2016 to February 2019. In all five sites (S1 to S5), for all five metals there were statistically significant variations of metal concentration in feathers of *Ardeola grayii*. Mn (17.8 ± 2.37) and Zn(48.17± 0.8) concentrations in feathers of *Ardeola grayii* were relatively higher, but mercury was not present any sample of feather. In the all five sites heavy metals present in order: Zn>Mn>Cr>Cd>. This study suggested that feather is used as non-invasive tool for metal concentration in feeding and breeding area of *Ardeola grayii*.

Key words : Heavy metal, feather, concentration, *Ardeola grayii*, habitat.

INTRODUCTION

Heavy metals are not always harmful for living organism, it plays vital role in lives of living species. In nature heavy metals present in trace levels in the structure of all living creatures, if these levels are exceeded shows noxious effect in the organism (Zolfaghari *et al*, 2007; Manjula *et al*, 2015). Metals like mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), cobalt (Co), and zinc (Zn) are highly toxic to all components of the ecosystem (Burger and Gochfeld, 1997; Gerbersmann *et al*, 1997; Lee *et al*, 2006) and it also shows severe impact on the environment and stability of ecosystem (Guven *et al*, 1999; Sanpera *et al*, 2000; Battaglia *et al*, 2003). Some metals are categorized into essential and non essential metals. Lead (Pb) is non-essential heavy metals for organisms and sources of lead are from burning of fossil fuels, road traffic and industries. It is indicator of metal pollution caused by anthropogenic sources by anthropogenic sources (Metcheva *et al*, 2006), including sewage disposal, fuel combustion, waste incineration, paints, vehicles, polishing, leather industry and oil spills (Jerez *et al*, 2011; Markowski, 2013). It is mostly present in calcareous tissues and in feathers of birds. Cadmium is also a non-essential element, present in the environmentas a result of anthropogenic activities, with detrimental effects for birds on egg formation, testicular damage, oviduct malfunctioning and kidney damage (Malik and Zeb, 2009; Burger, 2008; Eisler, 1985). When concentration of Cd is higher, it will interfere with replacement of essential elements at the active sites of biologically important biomolecules, thus indirectly impact on nutrients deficiencies (Furness, 1996). Lead and cadmium both are harmful for avian species (Scheuhammer, 1987), if it will intake through diet and directly influence the endocrine system (Stoica *et al*, 2000; Martin *et al*, 2003), kidneys (Nordberg, 1971), reproduction (Buerger *et al*, 1986), behavioral response (Hui, 2002), molting, migration (Honda *et al*, 1986), enzymes involved in hemoglobin formation and growth rates (Eisler, 1988). Chromium is not comes under essential elements for living organism and its impact on reproductive process of different avian (Malik and Zeb, 2009). Some of the study area is near leather industry, and the high concentrations of Cr can be related to the extensive use in tanning process of Cr salts, it was disposed in environment as effluent and sludge and finally it was entered in ecosystem via bioaccumulation from lower trophic level to higher trophic level through food chain (Khan *et al*, 2013). Mn is essential elements it involved in several biochemical reactions in animal as well as plants also, and it is essential micronutrient. Mn is generally present in igneous rocks, but due to uncontrolled traffic pollution, disposal of untreated wastages in environment, contributing Mn burdens in the environment (Zayed *et al*, 1999). Zn is also comes under essential elements it involved in many enzymatic system but sources of zinc used in alloying, dyeing, and manufacturing electric goods, insecticides and cosmetics (Irwin, 1997) and causes reduced species diversity and abundance, as well as fainting, nausea and stomach disorders (Perez-Lopez *et al*, 2008).

Birds plays important rolein ecosystem, they are having diverse and evolutionary successful species and occur in large number in tropics. They are indicator of environmental problems, and indication of healthy ecosystem. *A. grayii* forage on various habitats, where they prey on insects, annelida, amphibians, mollusc and fishes. In food web *A. grayii* is high on trophic level, they are useful as a bioindicator species for environmental pollution, especially aquatic environments. For sampling of feathers, feces and diet of *A. grayii* for metal analysis is known as non-invasive method. By observing concentration of metals in specific parts of sample determine which part is a mostly accumulated metal from which feeding areas of *A. grayii*. However, the feathers (Dauwe *et al*, 2003; Ek *et al*, 2004) were collected from breeding and feeding areas of *Ardeola grayii.* Herons are located in upper level of food web provides information on the status and condition of the related ecosystem (Burger, 1993). Metal analysis through feathers, feces, diet and habitats are non invasive and there is no any harmed to birds. In such way, it not only provide information on heavy metal concentration in *Ardeola grayii*, but also heavy metal concentrations in other living organism in lower trophic of the food web of it which are food of them (Battaglia *et al*, 2005).

Herons comes contact with these metals through soil, air and water, metals absorbed through the intestinal tract, circulate through the body, and deposited in different body organs, excreted directly or sequestered in feathers (Furness *et al*, 1986). Female birds excrete some metals in their eggs and eggshells (Fasola *et al*, 1998). Thus, monitoring environmental contaminants such as heavy metals in any area, birds are used as good indicator of not only health of their species but also fluctuation in environmental contamination where they live.

MATERIALS AND METHODS

Study area

The study was carried out in different areas of Lucknow city (upto 50 km) from September 2016 to February 2019, studied site was divided into 5; site 1, site 2, site 3, site 4 and site 5, all these sites were used by *Ardeola grayii* for breeding or feeding purposes.

Sample collection and preparation

Feather sample were collected in different areas of Lucknow, site 1 (Kallipaschim), site 2 (Mawaiya), site 3 (Behasa), site 4 (Deva) and site 5 (Unnao). Concentrations of six metals, namely, Cd, Cr, Pb, Hg, Mn and Zn were analysed in 18 samples from each sites. Feathers were collected from breeding and feeding habitats of *Ardeola grayii*, during sample collection we did not disturb the birds, white sheet spread behind nesting trees and fallen feathers collected from feeding sites.

Firstly, a minimum 2g of feather samples were washed 3 times with tap water, rinsed with distilled water, and then washed in acetone following, Saeki *et al* (2000) and Battaglia *et al* (2003) to get rid of the external pollutants (Goede and Bruin, 1984). One gram of feather sample was digested in a microwave digestion system using 10-ml concentrated nitric acid (HNO_3) acid for 10 min followed by 1 ml perchloric acid ($HClO_4$) for 5 min and 7 ml of hydrogen peroxide (H_2O_2) for 10 min at 250 W power (Muralidharan *et al*, 2004). Solutions were made up to 50 ml with deionized water. Duplicate samples were prepared using the same procedure. The digestion was carried out by using a hot plate with a magnetic stirrer, initially at low temperature and then at higher temperature. The digested samples were filtered, stored in amber vials and subjected for heavy metal residual analysis using atomic absorption spectroscopy. Prior to feather sample analysis, the instrument was calibrated using standard solutions of each metal obtained from Fisher Scientific Company.

Data analysis

SPSS (version 21.0) was used for the statistical analysis. Before using any statistical test first checked normality then according to distribution of data, test was used for further analysis. In all three year 2016-2019 concentration of metals for all five sites S1 – S5 statistically significant ($p > 0.05$), therefore, ANOVA and Kruskal – Wallis was used to determine the differences in metal concentration among all three year. Graph pad prism 5 was used to see variation in concentration of metals in studied sites, mean ± SD values are taken for graph.

RESULTS

Metal analysis through feather of *Ardeola grayii* is non-invasive tool and there is no harm to birds. The concentration of metals in all three year and variation in all five study sites in all five metals are detailed in Table 1 and Fig. 1. Metal analysis through feathers of *Ardeola grayii* was observed by few authors, so in this study non-invasive method was used for analysis of metals in feeding and breeding sites of it. Its feeding habits is diverse so variation can be easily observed.

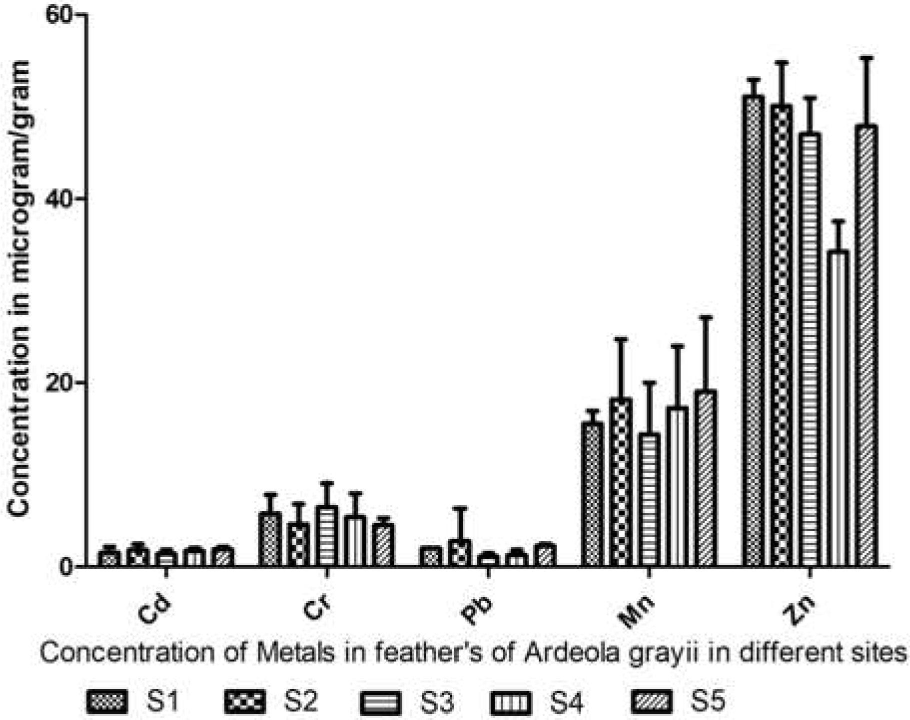

Fig. 1 : Concentration of heavy metals in feathers of *Ardeola grayii* in different sites.

Table 1 : Variation in concentration of heavy metals in feathers of *Ardeola grayii* in three year study period.

Heavy metals	2016-2017 (Mean±SD)	2017-2018 (Mean±SD)	2018-2019(Mean±SD)	F/H	p
Cd	1.19±0.44	1.79±0.37	2.04±0.26	20.633	0.00
Cr	4.80±2.24	5.46±2.24	5.79±2.11	0.794	0.45
Pb	1.27±0.72	2.40±2.73	1.98±0.36	1.823	0.17
Mn	10.56±3.34	17.21±2.91	22.81±4.21	34.40*	0.00
Zn	42.36±7.43	46.07±6.80	49.79±7.04	4.10	0.02

In this study, it was observed that for three year (2016-2019), there is statistically significant difference (Table 1) (p 0.01, $df_{2,42}$ F = 20.633), for chromium (p 0.02, $df_{2,42}$ F= 0.79), Pb (p 0.00, $df_{2,42}$ F= 1.82), Zn (p 0.00, $df_{2,42}$ F = 4.10) and Mn is not statistically significant (p 0.52, df_2, H=34.40). Thus, overall study revealed that in all three year metal concentration increased in all five sites.

Heavy metal concentrations (Cd, Cr, Pb, Mn and Zn) in feathers of *Ardeola grayii* in all five feeding sites comparison are shown by stacked bar charts illustrations with mean and standard deviations (Fig. 1). Heavy metal concentrations statistically significant ($p<0.05$) for all five metals and sites. *Ardeola grayii* feathers had metal concentration in order: Zn>Mn>Cr>Cd=Pb for all five sites for every year. The mean concentration of Cd recorded for all five sites are: (1.51± 0.6), (2.50 ± 1.02), (1.40 ± 0.44), (1.73 ± 0.33), (3.05± 1.17) respectively, *i.e*, S1 to S5. The concentration of chromium for all five sites are 5.75 ± 2.07, 6.91 ± 1.97, 3.22 ±1.77, 5.39 ± 2.59, 7.07 ± 1.56, respectively. The lead concentration detected in feathers of *Ardeola grayii* for all five sites are: 2 ± 0.03, 3.67 ± 1.58, 1.12 ± 0.33, 1.27 ± 0.56, 5.92 ± 1.88, respectively. The concentration of manganese in all five sites are: 15.53 ± 1.40, 18.18 ± 6.61, 14.33 ± 5.65, 17.22 ± 6.79, 23.73 ± 3.06. The concentration of zinc in all five sites are, 51.14 ± 1.8, 54.07 ± 2.44, 47.03 ± 3.96, 34.22 ± 3.32, 54.41 ± 3.57, respectively.

DISCUSSION

Biggest problem in whole world is environmental pollution, which resulted deterioration most of the ecosystem. It is especiallytrue for most developing countries in South Asia, such asNepal, India, Bangladesh, and Pakistan, where most of the wetlands are about to lost and water pollution increasing due to huge amounts of pollution load discharged by urban activities in water bodies. About 90% to 95% of alldomestic sewage and 75% of all industrial effluent are discharged into surface waters without any treatment. Lucknow is situated near Gomati river and its rural area have good number of agriculture land, but day by day due to increasing demand of land, most of agriculture field and wetlands are rapidly converting into building or industrial purposes, these all activities causes loss of flora and fauna. The main sources of environmental pollution are sewage water, domestic garbages, hospital discharges, fertilizers, pesticides, automobiles, industrial effluents and leather tanning. As a result, soils, air and water are heavily polluted with various heavy metals like Hg, As, Pb, Zn, Mn, Li, Cd and Cr (Abbas *et al*, 2004). Pesticides are major source of lead, cadmium, copper and chromium; also, there are glyphosate based herbicides that contain metals.

Sherer *et al* (2015), Manjula *et al* (2015), Kim and Oh (2012), Frantz *et al* (2012) have done work on avian feathers as biomonitoring tool of heavy metal pollution. Contaminated air, water, soil and food contribute to their accumulation in feathers, influence of other factors like habitat, food and feeding habits and proximity to human dwellings have by and large been ignored. Heavy metal analysis through feathers of birds is non-invasive techniques, there is no killing of any birds. In this study *Ardeola grayii* feathers are used for their feeding habitats monitoring heavy metals.

In this study, some areas are in urban and some in rural, site 1, 2 and 3 in urban, site 4 and 5 in rural. Site 2 and site 5 are polluted comparison to other three areas.

Concentration of cadmium is lethal for heron if concentration of is greater than 2μg/g dw in feathers. In avian feather accumulation of cadmium depend on food habits and habitat. In this study for all five sites feathers of *Ardeola grayii* have concentration below 2, but in site 5 it was higher (3.05ppm) than other four. Cadmium concentration in site 5 was due to tannery, automobile workshops. According to Burger (2008) and Scheuhammer (1987) cadmium is toxic to birds and it linked to egg formation, oviduct and malformation and kidney damage. Greater than 2μg/g dw of Cd in feather is associated with adverse effects such as kidney toxicity, eggshell thinning, lower food intake, disruption of calcium metabolism and intestinal damage in birds.

Chromium is not an essential element for animals, and it may cause deleterious effect on reproductive health of avian species (Malik and Zeb, 2009). Cr observed in feathers of *Ardeola grayii* for all five sites exceeding the threshold of 2.8μg/g (Burger and Gochfeld, 2000) could be anthropogenic source of contamination. Site 2 and site 5 have higher concentration of Cr, site 5 was near to tannery industry and effluent dumped into waterbody and site 2 was contaminated due to anthropogenic sources.

Lead is heaviest non-radioactive element and if ingested, it is poisonous to animals as well as birds. Sources of lead in the environment are lead pigment in paint, fuel additives, pipes and glazed ceramic food container etc. lead affects birds reproduction, behaviour and hematopoietic and nervous system. Concentration of lead greater than 4μg/g in feathers of herons is known to be potentially toxic and directly associated with chronic high level of Pb exposure (Burger and Gochfeld, 1997). Low level of lead is also danger for birds such as it affect growth of nesting, thermoregulation and individual recognition in some seabird (Burger and Gochfeld, 2000).

Mn is an essential micronutrient that plays a vital role in many metabolic and biochemical reactions. Mn contamination in food chain generally occur as a result of crustal origin, however certain other anthropogenic sources such as urban waste dumps and industrial effluents, vehicular traffic also contribute to Mn contamination (Abdullah *et al*, 2005).

Zn is an essential that plays important roles in different metabolic reactions, originate from both natural and anthropogenic sources, uncontrolled waste disposal in or near to water body.

In this study in all five sites zinc and manganese both present in higher concentration than other three metals, this is because of all these sites are badly disturbed by anthropogenic sources, no any proper disposal of wastage of any type.

CONCLUSION

This study result revealed that feathers of *Ardeola grayii* is used as a good biomonitoring tool for assessment of heavy metals, because it preferred both fishes as well as insects and may visit a variety of habitats with varying degree of contamination, this may result in the accumulation of more contamination. These contaminants not only affects birds, but also disturbed a whole ecosystem due to bioaccumulation. So this is matter of concern *Ardeola grayii* comes under least concerned but if such situation will continued its population will also decrease. Before such situation, we should take care of water bodies and proper disposal of wastage so that contamination not reaches to waterbody and aquatic birds or other organism can survive there.

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Seasonal variation in feeding behaviour and foraging success of Indian pond heron (Ardeola grayii) in different habitats

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Seasonal variation in feeding behaviour and foraging success of Indian pond heron (*Ardeola grayii*) in different habitats

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Abstract

Objectives: The present study was focused on feeding behaviour and foraging success of Indian pond heron (*Ardeola grayii*) in different seasons and habitats. **Methods:** The study was carried out in three habitats, lake, pond and marshy area from September 2016 to December 2017. All activities such as feeding behaviour, foraging success, prey abundance, success ratio, feeding frequency (foraging attempt and success) of *A. grayii* were compared in three habitats with three seasons by using binocular and video recorder. **Statistical analysis:** Data were analysed by SPSS (21 version) and graph pad prism. In this study, prey abundance and foraging success in three habitats and season differed statistically significant ($p < 0.05$). By using non-linear regression on foraging attempt and success in three habitats three curve (exponential, cubic, growth and power) best fitted to analyzed data sets. These curve shows variation in feeding pattern. **Findings:** Stand and wait is dominant feeding behaviour followed by walk slowly and walk quickly in all three habitats and season. Prey was abundantly present during monsoon, as a result feeding frequency and success ratio maximized. Structure of habitats, vegetation, and water depth also influenced foraging success of *Ardeola grayii*. Thus, overall finding showed that Indian pond heron feeding behaviour and foraging success affected by structure of habitats and seasons.

Keywords: Habitat; season; feeding; frequency; behaviour; prey

1 Introduction

Feeding is a crucial activity of the bird's life which is vital for its survival however the demands of food acquisition impose significant challenges to both physiology and behaviour of birds. The bird species, directly and indirectly, depends on different habitats for feeding, breeding, nesting and resting. Wading birds are most commonly associated with wetlands, streams, and other aquatic habitats such as ponds, lake etc. The members of family Ardeidae are medium to large wading birds, consist of 9 species viz.

Ardeola grayii (Indian Pond Heron), *Nycticorax nycticorax* (Black-crowned Night Heron), *Ardea cinerea* (Grey Heron), *Ardea purpurea* (Purple Heron), *Egretta garzetta* (Little Egret), *Bubulcus ibis* (Cattle Egret), *Mesophoyx intermedia* (Median egret), *Casmerodius albus* (Large egret) and *Ixobrychus cinnamomeus* (Chestnut Bittern). All those large wading birds captured prey by employing stand and wait for strategy (1; 2), or by walking slowly towards the prey and waiting for surprise attack (3).

Various feeding behaviour of *Ardeola grayii* were observed by many authors such as; walk slowly, surprise attack (3), fish baiting using bread crumps (4), scavenging behaviour (5), stand and wait (2), probing (6), floating behaviour (7). Feeding behaviour and feeding frequency of herons are affected by several factors, including; prey density and prey availability (8), seasons (9), habitat characteristics (10) and height of vegetation (11).

Seasonal variation in food abundance often influences the use of different habitats while seasonal rainfall changes the availability of food, geomorphology of habitats and seasonal crops (12; 13; 14). Seasonal variation in resource availability plays a dominating role in the evolution of species and communities (12; 13; 15). For most of the wading birds, critical seasonality is a wet and dry cycle of weather (16). Availability of aquatic prey may vary with seasons but this also depends on the fluctuation in water level condition. The availability of prey directly or indirectly affected by wet and dry conditions of weathers (16; 17), that can be a major limiting factor of avian populations (18; 19). Many studies have shown that prey availability within a habitat is important in determining wading birds' for selection of a foraging sites (20; 21; 22; 23). Besides the season and availability of prey, water depth can also affect both accessibility of foraging habitat and the vulnerability of prey to wading birds.

A. grayii is a long-legged wetland bird species and commonly known as Paddy bird. *Ardeola grayii* is generally fed alone or with small groups in all types of wetlands (5; 24) by its long bill. *Ardeola grayii* (Indian Pond heron) mainly forages on insects, amphibians, annelids, crustaceans and plant material on the ground (25) but during breeding preferred to feed on fishes (5). There are no ample studies available on the foraging ecology of Indian pond heron, only a few studies documented (26; 27). In India, most of the work was done on their breeding biology, but very few study carried out on foraging behaviour including feeding success, food items and feeding behaviour of *A. grayii*. Therefore this study aimed to access the effect of seasonal changes on foraging success and feeding behaviour of Indian Pond heron (*A. grayii*). Here tested variables for completing aim of this study are; (i) prey abundance in different feeding areas of *A. grayii* (ii) Prey abundance affect foraging success in different seasons, (iii) the foraging attempt and success (success ratio) in three habitats and season, (iv) Feeding behaviour in different habitats.

2 Methods

2.1 Study area

Field observations were carried out from September 2016 to October 2017 in the foraging habitats of *A. grayii*. The whole study period was divided into three seasons: monsoon (July - October), winter (November - February) and summer (March - June). The study was carried out in Lucknow (26.8470 N and 80.9470 E), and its associated areas (up to 50km). Three types of Wetlands Lake, pond and marshy area were selected, these all were manmade wetlands. According to Ramsar convention 2008, these wetlands were defined as Marshes which are periodically saturated flooded, or ponded with water characterized by herbaceous plants which fluctuate seasonally with wet and dry conditions. In this study site, marshy areas were flooded during rainy and sometime it dried out for grasses, rushes and low growing shrubs to flourish.

Lake is deep water body surrounded by woody and non-woody tree habitats of many flora and fauna. In this study site, the lake is surrounded by *Azadirachta indica, Eucalyptus globules*, *Mangifera indica, Vachellia nilotica, , Ipomoea carnea, Eichhornia crassipes, Pithecellobium dulce* etc.

Pond is shallow, permanent or semi-permanent water with little flow; in this study site, the pond is near to temple with many surrounding trees such as *Ficus benghalensis, F. virens, F. racemosa, Mangifera indica, Azadirachta indica* etc.

2.1.1 Feeding frequency (Foraging attempt /success)

Feeding activities of A. grayii was observed using binocular (Celestron Upclose G2, 6.8°/354FT/118m (10×50X) and digital camera (Sony and Nikon Coolpix). Feeding frequency of *A. grayii* recorded for three hours per day for five days every month without disturbing their activities. Feeding frequency was counted with a stopwatch and whenever possible through visually for each 15 min feeding bout (15-minute observation whether a prey species was captured by pond heron or not). A 15 min foraging attempt was considered successful if *A. grayii* caught any prey items, and was considered unsuccessful if it failed to do so. For calculating success ratio, foraging success was divided by foraging attempts (success/attempts) for every 15 minute of observation.

Success ratio = No. of foraging success/ No. of foraging attempt (for every 15 minute success and attempt was calculated)

2.1.2 Feeding behaviour

The different feeding behaviour of *A. grayii* was recorded for five days in every month, in this study feeding behaviour terms was followed by kushlan and many authors (3; 6; 7; 8; 16; 28; 29). Feeding behaviour such as a stand and wait, walk quickly, diving, bill dipping, crouched position, neck movement and Hopping were recorded. The recorded video was played back in slow motion and frequencies of every feeding behaviour were assessed for every 15 minutes.

2.1.3 Prey abundance

For the seasonal variation in prey availability, aerial prey and benthic prey were collected in foraging sites on all three study sites once in a fortnight. The prey species were identified and recorded. Prey items were sampled by 5 sweeps (30; 31) of a long-handled net from the water edge randomly between 0 and 5 meters, distance from water edge was measured by meter stick. Fishes were collected with the help of fisherman. Aquatic insects and fishes were identified following (31; 32) respectively. The size (length and weight) of prey items was measured with a scale, and released at the site of capture.

2.1.4 Statistical analysis

All statistical analysis was performed using SPSS (version 21.0). Normality and homogeneity were evaluated for the distribution of data sets. $P < 0.05$ was considered as statistically significant. Prey abundance of lakes, ponds and marshy area was found normally distributed ($p > 0.05$) except amphibians of lakes and annelids of ponds ($p < 0.05$). Data of foraging success of Indian pond heron in all three seasons in lakes and ponds for amphibians, annelids and molluscs for the lake, ponds and marshy area were found not normal ($p < 0.05$) while the rest of them were normally distributed ($p > 0.05$). Crouch position and Neck movements were found not normally distributed ($p < 0.05$) while the rest of feeding behaviour were found normally distributed ($p > 0.05$). For normally distributed data set ANOVA and for not normal data set Krushkal walis H-test were used to evaluate the differences in prey abundance, foraging success and feeding behaviour of *A. grayii* in three different habitats and seasons. We used curve fitted model to show different pattern of foraging attempt and success in different seasons and habitats.

3 Results

3.1 Prey abundance

In the current study, five types of prey items were recorded in three feeding habitats of *A. grayii* (Table 1). These three habitats comes under city area, and their distance to each other are as follows; distance between lake and marshy area is 4km, lake to pond 10km, marshy to pond 8km. Main prey items were insects (Belostomatidae, Argyroneta aquatica, Theatops californiensis Eurymerodesmus), annelids (Pheretima posthuma, Hirudo), fish (Channa punctata, Channa striatus, Clarias batrachus, Punctius chola, Chela atpar, Labeo rohita), Amphibians (Rana tigrina) and Mollusc (Snail). Insects were most abundant prey items (48%, 45% and 37% in lake, marshy and pond respectively), while of fishes were second most abundant (27%, 25% and 17% in pond, lake and marshy area respectively) in feeding habitats of *A. grayii*. Annelids (17%) and Molluscs (9%) were highest in marshy area followed by pond (12% and 10%) and lake (10% and 7%).

Prey abundance varies seasonally. Insects (34.58 ± 12.37), fishes (20.55 ± 6.54), amphibians (13.08 ± 7.31), annelids (14.58 ± 8.01) and molluscs (8.04 ± 3.19) were most abundant in monsoon in all three habitats, while lowest prey abundance was in winter (Table 1). There were statistically significant differences in prey abundance of pond in three different seasons ($p < 0.05$, Table 1). Insects ($F = 6.03$, $p < 0.05$), fishes ($F = 6.41$, $p < 0.05$) and molluscs ($F = 3.64$, $p < 0.05$) differed statistically significant for all three seasons in lake (Table 1), but in marshy area only fishes ($F = 4.53$, $p < 0.05$) and annelids ($F = 5.29$, $p < 0.05$) differed seasonally (Table 1).

Table 1. Prey abundance in three different habitats at different seasons

	Prey	Summer	Monsoon	Winter	F/H*	p
	Fishes	14 ± 5.97	19.25 ± 7.32	9.12 ± 2.74	6.445	0.007
	Insects	24.87 ± 8.99	37.37 ± 12.22	20.25 ± 9.05	6.031	0.009
Lake	Amphibians	7.62 ± 12.38	7.5 ± 6.50	2.75 ± 0.70	0.232*	0.63
	Annelids	5.875 ± 6.03	8.12 ± 2.74	3.62 ± 1.18	2.678	0.092
	Molluscs	4.25 ± 2.05	5.12 ± 2.41	2.62 ± 0.74	3.641	0.040
	Fishes	14.75 ± 4.97	19.75 ± 5.47	10.75 ± 2.76	7.826	0.003
	Insects	14.12 ± 3.48	31.12 ± 8.37	15 ± 4.07	22.254	0.001
Pond	Amphibians	5.37 ± 7.58	16 ± 5.09	3.12 ± 1.15	13.448	0.001

Continued on next page

Table 1 continued

	Prey	Summer	Monsoon	Winter	F/H*	p
	Annelids	4.5 ± 4.37	12.87 ± 7.45	2.5 ± 0.75	10.128*	0.006
	Molluscs	3.12 ± 3.64	9.75 ± 2.60	2.25 ± 1.03	19.107	0.001
	Fishes	7.75 ± 13.57	22.25 ± 6.84	4.75 ± 0.95	4.529	0.044
	Insects	23.5 ±10.27	35.25 ± 16.52	25 ± 9.48	1.047	0.39
Marshy	Amphibians	6.5 ± 11.70	15.75 ± 10.34	2 ± 0	4.891	0.037
	Annelids	6.5 ±11.70	22.75 ± 13.84	4.5 ± 0.57	5.298	0.03
	Molluscs	4.75 ± 3.09	9.25 ± 4.57	5.5 ± 0.57	2.262	0.16

* Kruskal Wallis-H Test

Over all abundance of prey in all three habitats did not differ significantly (Table 2) except insects in all three habitat (F = 3.2, p < 0.05).

Table 2. Over all prey abundance in three differenthabitats

Prey	Lake	Pond	Marshy		
Fishes	14.13 ± 6.85	15.08 ± 5.75	11.58 ± 11.27	2.941*	0.23
Insects	27.50 ± 12.23	20.08 ± 9.69	27.92 ± 12.55	3.2	0.048
Amphibians	5.96 ± 8.07	8.17 ± 7.65	8.08 ± 8.28	0.708*	0.702
Annelid	5.88 ± 4.16	6.62 ± 6.63	11.25 ± 11.61	3.128*	0.209
Mollusc	4.00 ± 2.08	5.04 ± 4.26	6.50 ± 3.55	4.822*	0.09

*KruskalWallis- H test

3.2 Foraging attempt and success (Success ratio) of *A. grayii*

Feeding success ratio of *A. grayii* differed seasonally. The feeding frequency was recorded in morning to evening in all three habitats of *A.grayii*. During summer (Figure 1A), in all three habitats it started feeding in morning hours the first peak of feeding was recorded at 8:00 A.M. While second peak at 11:00 A.M. and after 14:00 P.M. third peak of feeding was obtained and feeding continued till late in evening. In case of monsoon season (Figure 1B) first peak of feeding was obtained at 7:00 A.M. and at 8:00-11:00 A.M. Feeding activity continued i n same pattern neither increases nor decreases in all three habitats, second peak of feeding was recorded at 12:00 P.M. and third at 15:00 P.M. During winter (Figure 1C), there was more fluctuation than other two season, because of temperature and availability of food. First peak of feeding was recorded at 8:00 A.M. While second and third peak at 11:00 A.M. 13:00 P.M. respectively. After 13:00 P. M. feeding activity continued with low frequency in all three habitats.

To see if there were any relationship between foraging attempt and success of *A. grayii* in all three habitats and seasons, some models were used to described it. Out of 11 models (Tables 7) applied to identify for the attempt and success relationship of *A. grayii*, the best fit models were cubic, exponential, growth and power.

The exponential model was found the best fit in monsoon and winter for lake and pond, and marshy land in winter; initially the curve increased steeply and grows until it approaches stability attaining the asymptote.

The growth model (Table 7) was found to best fit model in summer season for marshy land and pond. In growth model curve, initial point of curve was lower, and with time foraging attempt and success of pond heron increasing and after a certain point, it stabilized. Growth curve represents that in mid of summer season, due to rainy started there was abundant prey available so the peak of the curve was reached a maximum in mid-point. The cubic model (Table 7)was found to best fit model in summer season for the lake. In cubic curve, itcurve was in stationary phase and after 13:00 there was exponential growth.Power model was found to best fit model in monsoon season in marshy land;in power curve, there was a proportionate increase in both variables (foraging attempt and success).

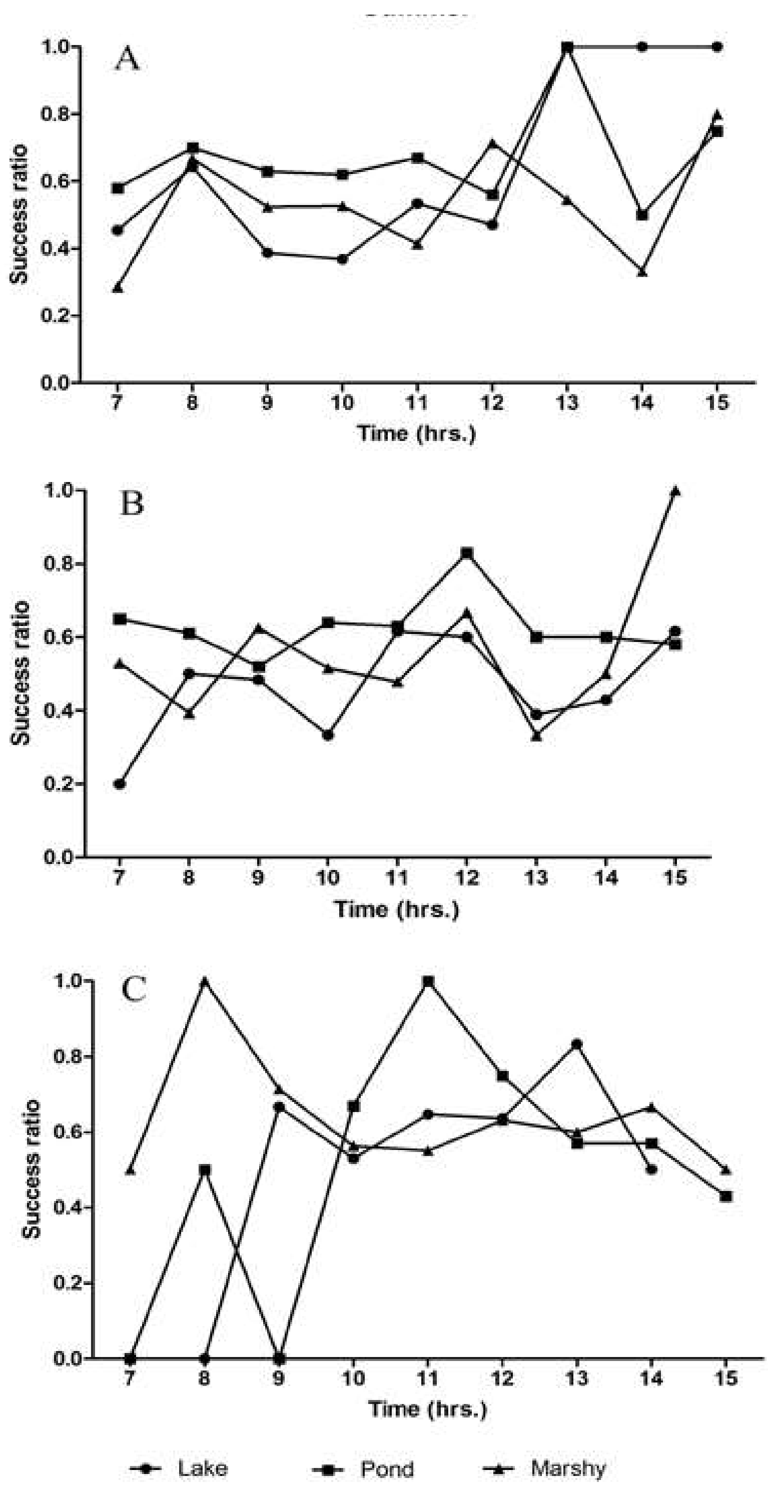

Fig 1. Represents success ratio of *A. grayii* during Summer (A), Monsoon (B), and Winter (C) seasons

3.3 Foraging success with reference to prey abundance

Foraging success of any birds depend on selection of habitats and prey. But some factors also influence its activity such as foraging ground, vegetation, and depth of water which influence the foraging success of *A. grayii*. It was observed that it preferred to feed near edge of water body or if any aquatic vegetation such as water hyacinth present then perched on that for feeding. *A. grayii* preferred habitat if water depth was 10-17cm, that's why it never feed in flooded water body. The structure of foraging habitat is also a determining factor for their foraging success, habitats rich with trees, shrubs, grasses mostly choosed for their daily activities.

The foraging success was highest in pond (33.60 ± 29.30) followed by lake (31.87 ± 30.85) and marshy area (11.13 ± 11.61). Foraging success also differ seasonally, it was observed that foraging success of *A. grayii* maximum in monsoon and lowest in winter in all three habitats (Table 3). The current study revealed that foraging success of *A. grayii* differed significantly for fishes ($F = 9.82$, $p < 0.05$), insects ($F = 27.82$, $p < 0.05$), amphibians ($H = 17.28$, $p < 0.05$), annelida ($H = 13.06$, $p < 0.05$), and mollusc ($H = 8.32$, $p < 0.05$) in ponds (Table 3), while in lake fishes ($F = 5.07$, $p < 0.05$) and insects ($F = 4.15$, $p < 0.05$) and in marshy fishes ($F = 4.03$, $p < 0.05$) and annelids ($F = 5.39$, $p < 0.05$) differed significantly (Table 3).

Table 3. Foraging success of Indian pond heron in three different habitats and seasons

Habitats	Prey	Summer	Monsoon	Winter	F/H	p
	Fishes	4.75 ± 3.01	7.25 ± 3.53	2.75 ± 1.58	5.068	0.016
	Insects	9.75 ± 3.10	13.37 ± 4.56	8.12 ± 3.35	4.153	0.03
Lake	Amphibians	1.87 ± 2.47	2.62 ± 3.12	1 ± 0	3.844 *	0.146
	Annelida	1.87 ± 1.80	1.87 ± 1.12	1 ± 0	4.519*	0.104
	Mollusca	1.12 ± 0.35	1.37 ± 0.74	1 ± 0	2.277*	0.32
	Fishes	2.75 ± 4.85	7.75 ± 3.59	1 ± 0	4.034	0.05
	Insects	8.75 ± 4.78	13 ± 5.71	9.5 ± 4.79	0.786	0.485
Marshy	Amphibians	2.5 ± 4.35	5.25 ± 3.77	1 ± 0	3.775	0.065
	Annelida	2.25 ± 3.82	8.25 ± 5.18	1 ± 0	5.394	0.029
	Mollusca	1 ± 0.81	1.5 ± 1	1 ± 0	1.114*	0.573
	Fishes	6.5 ± 2.87	9.25 ± 2.91	3.62 ± 1.59	9.818	0.001
	Insects	4.87 ± 1.95	13.87 ± 3.52	5.12 ± 2.53	27.823	0.001
Pond	Amphibians	1.87 ± 2.47	5.37 ± 3.20	1 ± 0/	17.285*	0.001
	Annelida	1.62 ± 1.40	5 ± 4.10	1 ± 0	13.057*	0.001
	Mollusca	1.6 ± 1.34	2.37 ± 1.50	1 ± 0	8.316*	0.016

*Kruskal wallis H-test

When we compare foraging success of *A. grayii* for prey items as: insects, fishes, amphibians, annelids and molluscs in three different habitats, we found that foraging success of *A. grayii* only differs in case of fishes ($H = 6.49$, $p < 0.05$, Table 4).

Table 4. Foraging success of Indian pond heron in different habitats

Prey	Lake	Pond	Marshy	F/H	p
Fishes	4.92 ± 3.29	6.46 ± 3.37	3.83 ± 4.34	6.488*	0.03
Insects	10.42 ± 4.21	7.96 ± 5.01	10.42 ± 5.01	1.963	0.15
Amphibians	1.83 ± 2.31	2.75 ± 2.95	2.83 ± 2.76	4.021*	0.13
Annelida	1.58 ± 1.24	2.54 ± 2.99	3.92 ± 4.42	3.088*	0.21
Mollusca	1.17 ± 0.48	1.70 ± 1.26	1.25 ± 0.62	2.663*	0.26

*Kruskal wallis H test

3.4 Feeding behaviour of *A grayii*

In the current study, nine types of feeding behaviour of *A. grayii* recorded in different seasons and habitats : stand and wait (SW), walk slowly (WS), walk quickly (WQ), crouched position (CR), diving (DIV), bill dipping (BD), neck movement (NM), hopping (HOP) and probing (PROB). We found that stand and wait was frequently used behaviour followed by walk slowly, walk quickly, crouched position, diving, bill dipping, neck movement, hopping and probing at different habitats (Table 5). *Ardeola grayii* did not used bill dipping in marshy and probing in pond. Stand and wait (SW) (F = 5.25, $p < 0.05$) and bill dipping (BD) (F = 3.18, $p < 0.05$) statistically differed in all three habitats (Table 5), while WS, WQ, CR, DIV, BD, NM, HOP and PROB showed no significant differences in lake, pond and marshy area ($p > 0.05$, Table 5).

Table 5. Variation in feeding techniques of Indian pond heron at different habitats

Behaviour	Lake	Marshy	Pond	F/H	p
SW	34.17 ± 6.87	25± 12.18	36.08 ± 6.68	5.25	0.01
WS	7.75 ± 2.7	6.17 ± 4.17	6.33 ± 4.22	0.64	0.53
WQ	5.42 ± 2.02	4.50 ± 2.9	4.33 ± 2.96	0.57	0.56
CR	2.33 ± 1.15	2.33 ± 1.61	2.67 ± 2.01	0.19	0.90
DIV	4.0 ± 1.80	3.0 ± 1.63	2.75 ± 1.81	1.53	0.23
BD	4.08 ± 2.15	1± 0	3.17 ± 2.36	3.18	0.05
NM	9.08 ± 9.34	3.67 ± 4.57	4.42 ± 3.57	5.45*	0.06
HOP	3.50 ± 2.17	2.33 ± 1.43	2.83 ± 1.52	1.04	0.36
PROB	3.50 ± 2.87	3.33 ± 2.22	1 ± 0	2.61	0.09

*Kruskal wallis H-test

In lake, *A. grayii* showed significant differences in hopping (F = 20.63, $p < 0.05$) and probing (F = 14.54, $p < 0.05$) for three season (Table 6), in all three season, hopping was least used feeding techniques in winter (1 ± 0), followed by summer (2.75 ± 1.70), and monsoon (6.75 ± 2.06), probing was used only in summer (2.75 ± 1.70), and monsoon (6.75 ± 2.06). In marshy, walk slowly (F = 4.96, $p < 0.05$), neck movement (H = 8.89, $p < 0.05$), hopping (H = 7.85,$p < 0.05$), and probing (F =5.54, $p < 0.02$) differed significantly in three seasons. In marshy area, diving was used in monsoon season not in other two seasons. In pond, stand and wait (F= 4.58, $p < 0.05$), diving (H = 6.09, $p < 0.05$), and bill dipping (H = 9.35, $p < 0.05$) differed significantly in all three season. Overall results showed that in all three habitats and seasons, stand and wait was dominant feeding techniques used by *A. grayii*, these were depended on type of prey and structure of habitats.

Table 6. Seasonal variation in feeding techniques of Indian pond heron at different habitats

Habitats	Behaviour	Seasons			F/H	p
		Summer	**Monsoon**	**Winter**		
Lake	SW	29.75 ± 2.06	35.50 ± 9.846	37.25 ± 5.56	1.39	0.29
	WS	6.25 ± 3.30	8.50 ± 3.10	8.50 ± 1.29	0.91	0.38
	WQ	4.25 ± 1.25	6.25 ± 2.36	5.75 ± 2.21	1.07	0.38
	CR	2 ± 0.81	2 ± 0.81	3 ± 1.63	1	0.40
	DIV	4 ± 2.16	4.75 ± 1.70	3.25 ± 1.70	2.44	0.10
	BD	3.50 ± 3.10	5.50 ± 0.57	3.25 ± 1.70	1.41	0.29
	NM	11.75 ± 10.93	12 ± 11.34	3.50 ± 3.10	1	0.37
	HOP	2.50 ± 1.29	6.75 ± 1.70	1.25 ± 0.50	20.63	0.001
	PROB	2.75 ± 1.70	6.75 ± 2.06	1 ± 0	14.54	0.002
Marshy	SW	38 ± 6.37	31 ± 4.54	39.25 ± 7.04	2.13	0.17
	WS	2.25 ± 2.50	9.0 ± 4.39	7.75 ± 2.36	4.96	0.03
	WQ	2 ± 1.5	6.50 ± 2.88	5 ± 2.16	3.7	0.06
	CR	2.25 ± 1.89	2.50 ± 1.91	2.25 ± 1.50	0.02	0.97
	DIV	-	3 ± 1.63	-	-	-
	BD	-	3 ± 1.63	-	-	-
	NM	1 ± 0	1.25 ± 0.50	8.75 ± 4.99	8.89*	0.01
	HOP	1.75 ± 0.95	4 ± 0.81	1.25 ± 0.50	7.85*	0.02
	PROB	1.75 ± 0.95	5.50 ± 2.08	2.75 ± 1.70	5.54	0.02
Pond	SW	34 ± 2.94	27 ± 12.49	14 ± 10.23	4.58	0.04
	WS	8.25 ± 3.86	7.75 ± 4.03	2.5 ± 2.38	3.3	0.08
	WQ	6.50 ± 3.10	4.50 ± 2.64	2 ± 1.41	3.26	0.08
	CR	4.0 ± 2.82	2.50 ± 1.29	1.50 ± 1	1.78	0.22
	DIV	4.25 ± 0.97	2.75 ± 2.21	1.25 ± 0.50	6.09*	0.04
	BD	5.50 ± 1.73	3 ± 2	1 ± 0	9.35*	0.01

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Table 6 continued

Habitats	Behaviour	Seasons			F/H	p
	NM	3.75 ± 1.25	6.25 ± 5.96	3.25 ± 1.70	0.77	0.49
	HOP	4 ± 1.82	2 ± 0.81	2.50 ± 1.29	2.29	0.15
	PROB	-	-	-	-	-

Table 7. Seasonal variation in feeding frequency of (*A. grayii*) Indian pond heron at different habitats

Habitat	Seasons	Model	S2
	Summer	Cubic	1.273
Lake	Monsoon	Exponential	5.439
	Winter	Exponential	2.428
	Summer	Growth	2.084
Pond	Monsoon	Exponential	3.240
	Winter	Exponential	0.504
	Summer	Growth	1.114
Marshy land	Monsoon	Power	5.870
	Winter	Exponential	3.253

S^2 = Residual sum of square

4 Discussion

4.1 Prey abundance

In the current study *A. grayii* used lake, pond and marshy area for feeding activity. Insects were the most abundant prey items, while fishes second most abundant in all three habitats of *A. grayii*. Fishes and amphibians were highest in ponds while insects, annelids, and molluscs were in the marshy area. All three habitats having different structures therefore availability of prey items also varies (33). During summer prey availability decreases in lake, but as rainy starts availability of prey increases (34). Thus the availability of prey in the lake is maintained in every season. In the pond, mainly fishes are available in all seasons rather than other prey items. In all three habitats, the availability of all preys such as insects, fishes, annelids amphibians and molluscs were highest in monsoon (35), while lowest in winter. The availability of prey depends on both structure of habitats and seasons (36; 37). During monsoon (36; 38; 39), most of the fishes, amphibians, annelids and aquatic insects are in reproductive phase, so their number increases in all types of habitats (40), which guarantee a relatively high abundance of potential prey for *A. grayii* (27). The marshy area after winter dries rapidly and becomes inadequate for feeding site for *A. grayii*. These results suggest that prey diversity increased in number during monsoon, so all prey items were present abundantly while in summer and winter insects and fishes mainly present in all habitats.

4.2 Foraging attempt and success (Success ratio) of *A. grayii*

Prey abundance, the structure of habitats, variation in seasons determined the success ratio of *A. grayii* **.** In the summer season, due to high temperature all wetlands having scarcity of water, so there was less availability of prey, as a result, success ratio graph showed temporal variation in every hours of observation for every season. In summer, birds come in feeding ground early in the morning while in winter late in morning, and in summer feeding continued till evening. In monsoon, *A. grayii* having a diverse diet, because in monsoon it is a reproductive period for amphibians, insects, Annelida, and fishes. So their number was higher as a result success ratio of *A. grayii* was higher. In monsoon, foraging success did not only depend on prey abundance but also water level. If the water level was not approachable to *A. grayii*, it did not feed; as the water level goes down it started to feed. In winter, as temperature decreases very few preys available, so *A. grayii* success ratio decreases.

Exponential curve showed feeding pattern of *A. grayii* in winter and monsoon season in all three habitats. Exponential curve pattern shows in starting of winter foraging attempt and success was increased but as temperature decreases, availability of prey decreased as a result foraging frequency decreased. In case of monsoon. The availability of prey abundance increased but *A. grayii* was not easily approached to prey on them so initially there was low frequency of feeding, but as water level goes down the feeding frequency again raised.

Growth curve represents the feeding pattern of *A. grayii* in summer season in both pond and marshy land. Such type of pattern of curve was due to availability of prey in habitats, in summer. The starting of summer season is the starting of breeding period of *A. grayii*. Most of the time, it was observed busy in searching suitable place for nest construction, so the feeding frequency becomes low. But as the part of breeding is over, its feeding frequency gradually increases (27).

The cubic curve showed the feeding pattern of *A. grayii* in the lake during the summer season. The cubic curve showed the initial frequency of feeding as slow but after sometimes it increases. In summer, the availability of prey was not abundant as monsoon season, but the requirement of energy for *A. grayii* was high in summer due to breeding period so the foraging attempt in that case increased. Power curve represents the feeding pattern of *A. grayii* in marshy land during monsoon season. In this curve, both foraging attempt and success is in equal proportion due to availability of prey abundantly.

4.3 Feeding behaviour of *A grayii*

A. grayii varied their feeding techniques according to habitats (33) along with several environmental factors such as water depth (40) , the height of vegetation (41; 42), and availability of prey (40). *A. grayii* mainly used the stand and wait technique in all three habitats. However, in addition to stand and wait, *A. grayii* also adopted the walking slowly, similar techniques also adopted by Great egrets and Little egrets (5; 43; 44). *A. grayii* used walk slowly for capturing sedentary or slow-moving prey in shallow water, marshy and vegetated area (6; 43; 45). Stand and wait is better for capturing large prey in deep water or for finding hidden prey (46). Crouched position, hopping and probing was very least preferred techniques by *A. grayii* for foraging in all three habitats. Time spent in Stand and wait technique differ in ponds, lakes and marshy area, while time spent in other techniques did not differed in all three habitats. *A. grayii* did not use bill dipping in marshy and probing in the pond. Feeding techniques used by *A. grayii* did not varied with seasons, only hopping (HOP) and probbing (PROB) varied in lake and marshy while diving (DIV) and bill dipping (BD) in ponds.

In this study, it was observed that pond was generally choosen during breeding period by *A. grayii*, because in that period it had to construct nest, feed male to female, female to male during rearing, and chick also. So near pond many trees were available for construction of nest, and fishes (5) available as food. That's why near pond mostly these three techniques (stand and wait, diving and bill dipping) used by them. The present study indicates that prey availability, habitat structure, feeding techniques, all depend on variation in season, which ultimately affects feeding activities of *A. grayii*.

5 Conclusion

In this study, it was concluded that *A. grayii* employs different behaviour for feeding, but stand and wait was dominant feeding behaviour. Insects were present abundantly in all three habitats. So foraging success for insects were higher than other prey items. But during summer and starting of monsoon fishes were mostly preferred by *A.grayii*. Monsoon season was the favourable season for feeding of herons due to availability of prey. So in all three habitats, foraging success was higher in monsoon. In this study it was observed that insects were mostly consumed prey by *A. grayii*. So it plays a significant role in controlling the population of insects and other invertebrates on which they fed and supports the farmers. However, the rapid urbanization and development of the area for living and other human purposes may create a threat to these birds by the destruction of natural habitats through anthropogenic activities. Thus, if any disturbances occur in habitats of *A. grayii*, its feeding activity will affect; as a result, their breeding activity will hamper and finally decrease their populations. Not only *A. grayii* but all wetlands dependent birds will also be affected. Thus proper conservation the wetland bird species is important for the conservation of entire ecosystem.

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Original Research Article

Analysis of Heavy metals and Physico-chemical Parameters of Water in Feeding Habitats of *Ardeola grayii*

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ABSTRACT:
The aim of this study was to determine the levels of some physico-chemical parameters of water from feeding sites of *Ardeola grayii*. Water samples were collected from five feeding sites of *Ardeola grayii* as site 1 to 5 for the determination of BOD, COD, DO pH, Chloride, Alkalinity, Total hardness as calcium carbonate, and magnesium carbonate. Samples were also collected for determination heavy metals such as; Cd, Cr, Pb, Hg, Mn, and Zn. Statistical analysis was done by SPSS and Graph pad prism 5. The concentration of DO, BOD, COD was higher in site 2 and site 4. As a result that contaminated water was lethal for aquatic organism and directly or indirectly it affects *Ardeola grayii*. The concentration of Cd, Pb, and Hg was higher in site 2, 3 and 4 than CPCB limits indicating severe contamination in these sites. Due to presence of these anthropogenic substances, *Ardeola grayii* shifted their feeding habitats and behaviour, which is not good for ecosystem. On the contrary, if such pollutants continue to occur for a longer then it may leads to the extinction of rest of the species. Monitoring should be continuously done in order to alleviate pollutants and maintain proper food chain of these aquatic ecosystems.

Keywords: Physico-chemical properties, Heavy metals, Behaviour, Pollutants

INTRODUCTION

Wetlands are the major players in maintaining water cycle and habitats for all wading birds. It provides breeding sites and resting ground for many birds and also inhibits aquatic insects and fishes. Aquatic insects that are major component of wetland ecosystem, providing good sources of food for, fishes and for *Ardeola grayii*, thus a making a complete food chain in an ecosystem. Wetlands are also used by human long ago as aquaculture for rearing prawn, fishes. There has been a strong relationship between human activity and disturbance of the aquatic environment (Hodkinson et al. 2005).

Wading birds and aquatic organisms are dependent for their daily activities on water bodies, and it has the ability to detect, discriminate, and respond to the pollutants and are also sensitive to both beneficial and harmful chemicals. Recent findings have proposed that deterioration of water quality is because of excess acidification and the presence of nitrogen,

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phosphorus, heavy metals, organic toxicants, and pesticides (Bloxham et al. 1999). Pollutants introduced to the environment have impact on ecosystems, and is found in the whole biosphere.

Physico-chemical contamination may affect ecosystems, causing changes in the functions of particular organisms (Bhat et al. 2009). Lucknow the capital of Uttar Pradesh situated on the bank of the river Gomati, which is the habitat of faunal diversity. Unfortunately due to intense colonization, discharge of harmful industrial wastes, cutting of trees, conversion of agriculture field into buildings, its air and water quality are not healthy and sometime air quality index reached to 493 (CPCB), water reservoirs has severely impacted (Canpolat and Calta, 2001).

Ardeola grayii can be easily seen in both urban and rural area but due to decreasing waterbody in urban area of Lucknow, its population is decreasing rapidly.

In the present study*, Ardeola grayii* is used as indicator for habitat quality. Birds are considered good bio- indicators on habitat quality and the effectiveness of ecosystem, providing services to ecosystem and humans, as well as a good indicator of pollution and biodiversity. They are one of the connecting links of the food chain in the ecosystem and having important role in their habitats. There is reciprocal relationship between environment and birds. Environment provide essential factors such as resting, breeding, feeding, ground for their survival while birds contribute direct and indirect role in maintaining environment or modifying certain environment components (Ried, 1991 ; Block and Brennan 1993). In addition, birds are considered as an excellent communication means to raise awareness of biodiversity issues in a way that many organisms cannot (Gregory and Strien, 2010).

Water pollution is becoming a big problem for biodiversity. The main source of water pollution are industrial waste, domestic waste, sewage wastage which directly flow in water body and acid rain is also contribute in water pollution which cause deposition of heavy metals in waterbodies Obasohan et al., 2008.

Fresh water is a source for the development of civilizations but due to pollution there is severe threat to natural fresh water reservoir (Benjamin et al., 1996). The impairment of water quality due to introduction of pollutants is a problem faced by most industrial cities around the world. Rapid urbanization and industrialization with improper environmental planning often lead to discharge of industrial and sewage effluents into wetlands. The wetlands have a complex and fragile ecosystem, as they do not have a self-cleaning ability and therefore readily accumulate pollutants.

Heavy metals entering the water body would be adsorbed in sediments, and subsequently might migrate as a result of exchanges between water, sediment, and biota, through biological and chemical process. Heavy metals do not degrade in water but are generally not found in high concentrations, primarily due to deposition in sediments but also because of uptake by aquatic organisms. Birds are exposed to heavy metals through air, water and their food. Once a metal has entered the body it can be stored or accumulated, or be excreted (Dauwe et al. 2000).

Heavy metals impact on metabolic and reproductive ecology of birds. Water quality influences the availability and accessibility of prey items to various aquatic predators. The water quality is important in waterbird habitat assessment because a host of interacting physical and chemical factors can influence the level of primary productivity in aquatic systems and thus influence the trophic structure and total biomass throughout the aquatic food web (Wetzel 1975).

The physico-chemical characteristics of the water largely determine the waterbird community of wetland habitats, primarily by their direct and indirect impact on the availability and abundance of the birds' prey (Nagarajan & Thiyagesan 1996).

The physico-chemical environment can also directly and indirectly affect waterbirds daily activities. In a direct way, for example, different species of shorebird are constrained morphologically to forage at specific water depths (Safran et al. 1997).

Indirectly, however, physiochemical variables such as salinity and acidity affect the distribution and richness of benthic invertebrates (Courtney and Clements 1998, Leland and Fend 1998, McRae et al. 1998), which in turn can affect the feeding ecology of waterbirds.

So due to all these pollutants either wastage dumped in water or metals present in trophic levels causes variations in the water bodies which disturb the biodiversity (Odum et al., 1971). These variations such as pH, BOD, COD, DO, Chloride, hardness, calcium carbonate, magnesium carbonate, alkalinity in the freshwater bodies. The aim of the present study was to discuss physico-chemical parameters and heavy metals present in water, sample collected from feeding ground of *A.grayii*. This study were divided into three parts, in first and second part physico-chemical parameters and heavy metals of water analysed, and in last contaminated area and less contaminated feeding habitats of *A.grayii* was observed to see there was any different or unique behaviour showed by it. Behaviours were observed during feeding and resting time of *A.grayii*.

MATERIALS AND METHODS

In order to analyse physico-chemical and heavy metals in wetlands, water samples were collected in twice a month in replicates in urban and rural area of lucknow from September 2016 to February 2019, in pre-cleaned polyethylene bottles. The surface water samples were thoroughly filtered through cellulose nitrate filter paper to eliminate suspended solids and stored in plastic bottles with one liter capacity. For the measurement of dissolved oxygen (DO) and biological oxygen demand (BOD), separate 300 ml clean glass stopper BOD bottles were used for sample collection (standard volumetric Winkler's method). For metal analysis, 5 ml nitric acid was immediately added after collecting the samples, for heavy metal samples were collected separately. Digested samples were placed in pre-washed polyethylene bottle, various standards of heavy metals were prepared from certified standard stock solution (ppm) by using double distilled water. These standards were used to obtain calibration curve on Atomic Absorption Spectrophotometer. Effect of pollutants and changes in physico-chemical parameters of water on *A.grayii* behaviour was also observed. Water samples were analyzed for heavy metals (Pb, Cd, Cr, Hg, Zn and Mn) in Atomic Absorption Spectrophotometer. All parameters and procedures followed from CPCB 2008, and APHA 2005. Normality of data were analysed through SPSS (version 21.0) for every year data, mean ±SD values were taken, and graph created by Graph pad prism 5.

RESULTS

In this study it was observed that the polluted wetlands were generally occupied by *A.grayii* cattle egrets, and moorhen other wading birds were little in number, But due to very few availability of prey in polluted wetland, *A. grayii* had to wait for longer and observed to frequent changes of feeding positions and foraging patches.

Physico-chemical parameters:

The physico-chemical environment of water functions in many ways and employs the influences upon biotic components, thus, giving a picture of the environmental suitability of water to maintain life (Kumar and Singh, 2002). Temperature affects various chemical and biological reactions taking place in water and aquatic organisms (Shrivastava and Patil, 2002) and depends upon the season, time of sampling and also upon the temperature of effluent which is being added into the river. The values of different physico-chemical parameters of the water of different area of lucknow (upto 50 km) from all the samplings points during Sep 2016 to Feb 2019 are given in Table 1, 2, and 3. The values are the mean ± SD values of observation from all the 5 sampling points. In this study it was observed that how water quality affects *Ardeola grayii* foraging activity, and mostly affected thing was diversity of aquatic birds which depend on waterbodies for feeding. The mean variations (Mean±SD) of the water physico-chemical parameters for the 3-year study periods are given in the Tables 1, 2 and 3, respectively. In this study 3 year observation was done, in 2016-2017 in all five habitats pH was ranges between 6.3 – 6.9, too much fluctuation in pH are stressful and can even be lethal to aquatic organisms, which may circulate in a food chain. Levels of pH too high (> 9) or too low (< 5) can kill aquatic life (Younos 2007).

Table1: Variation in Physico-chemical parameters of water in different feeding habitats of *Ardeola grayii* from 2016-2017 (all values are given in Mean±SD)

Physico-chemical parameters	S1	S2	S3	S4	S5
pH	6.3±0.15	6.5±0.3	6.4±0.1	6.9±0.15	6.6±0.37
BOD	23.76±1.49	29.03±1.00	14.4±0.60	5.06±0.51	24.73±1.45
COD	23.19±0.45	27.82±0.20	18.38±0.47	14.69±0.37	26.88±0.32
Chloride	50.1±0.1	59.8±0.62	22.10±0.20	36.2±0.15	31.93±0.20
Dissolved oxygen	6.3±0.20	4.3±0.20	6±0.1	7.1±0.2	7.36±0.15
Total hardness calcium as calcium carbonate (mg/l)	165.1±0.1	311.36±1.18	294.6±4.16	144.2±1.08	137.7±8.63
Calcium as calcium carbonate	29.6±0.45	46.1±0.1	33±0.78	55.4±0.4	50.63±0.37
Magnesium as magnesium carbonate	22.1±0.2	47.3±0.20	47.21±0.26	60.7±0.61	32.13±0.90
Alkalinity	103.03±1.76	361.7±0.60	255.3±1.18	111.46±1.28	323.2±0.26

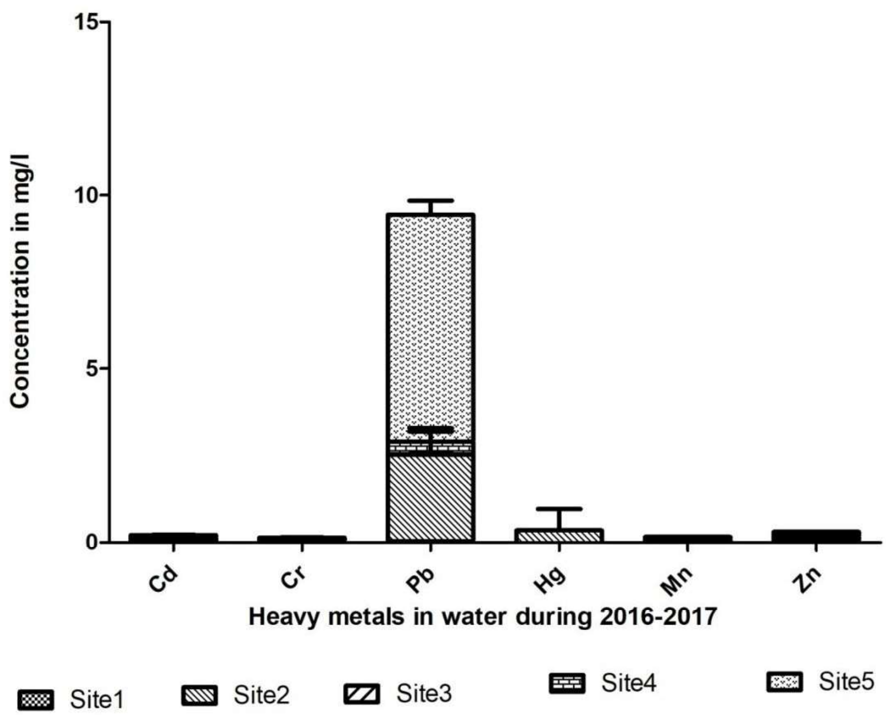

Figure 1: Heavy metals present in water for all five feeding sites of *Ardeola grayii* in 2016-2017

Table 2: Variation in Physico-chemical parameters of water in different feeding habitats of *Ardeola grayii* 2017-2018 (all values in Mean ±SD)

Physico-chemical parameters	S1	S2	S3	S4	S5
pH	6.3±0.32	7.8±0.25	7.23±0.15	7±0.1	6.9±0.41
BOD	27.43±1.06	8.9±0.6	16±0.1	35.1± 0.1	27.7±0.55
COD	29.7±0.5	41.8±0.5	32.8±0.37	34.43±0.30	32.3±1.10
Chloride	43.3±1.07	54.16±1.5	20.83±0.6	42.2±0.9	40.7±0.25
Dissolved oxygen	5.5±1	4.6±0.50	5.03±0.15	6.1±0.35	5.46±0.35
Total hardness calcium as calcium carbonate (mg/l)	167.9±0.05	308.7±2.3	299.8±0.72	167.83±0.40	308.7±2.3
Calcium as calcium carbonate	28.3±0.46	44.83±0.5	33.1±0.3	32±0.69	60.9±0.7
Magnesium as magnesium carbonate	21.34±0.9	47.16±1.35	47.3±0.26	44.7±0.55	56.5±0.39
Alkalinity	106.7±2.3	357.5±6.5	298.4 ± 2.4	166.43±1.64	319.8±0.66

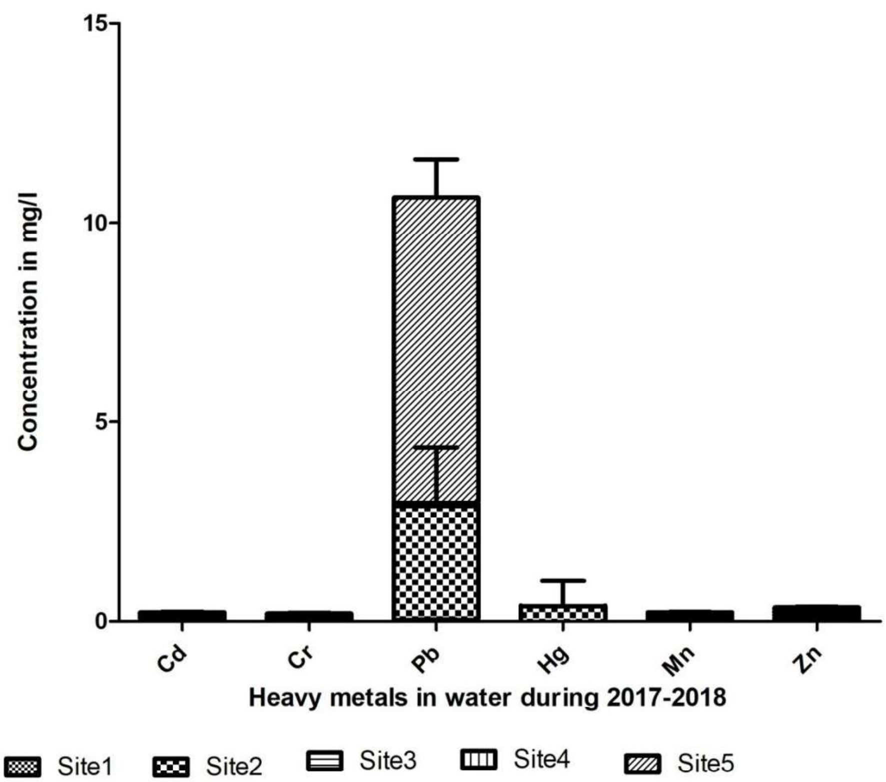

Figure 2: Heavy metals present in water for all five feeding sites of *Ardeola grayii* in 2017-2018

Table 3: Variation in Physico-chemical parameters of water in different feeding habitats of *Ardeola grayii* 2018-2019 (all values are given in Mean±SD)

Physico-chemical parameters	S1	S2	S3	S4	S5
pH	7.06±0.15	8.1±0.26	7.03±0.15	7.5±0.36	8.03±0.15
BOD(mg/l) 3 days at 27°C	27.3±1.70	12.5± 0.20	17.2±0.9	43.2±2.74	41.3±1.21
COD(mg/l)	24.7±0.50	60.7±2.08	33.3±0.9	31.4±4.3	34.3±1.1
Chloride (mg/l)	44.7 ±0.5	62.1±1.90	22.7±0.5	28.6±0.7	45.3±0.25
Dissolved oxygen	7.3±0.20	3.8±0.25	6.2±0.15	5.3±0.47	4.56±0.32
Total hardness as calcium carbonate	155.3±1.01	333.9±11.1	266.8±11.5	176.1±0.86	351.06±5.5
Calcium as calcium carbonate	26.4±1.15	45.16±4.3	36.9±0.68	41.6±1.37	61.46±1.5
Magnesium as Magnesium carbonate	25.27±1.00	50.5±1.70	40.2±1.11	57.9±0.62	51.5±0.6
Alkalinity	113.7±5.8	322.13±4.3	295.7±4.2	181.9±0.78	311.5±1.22

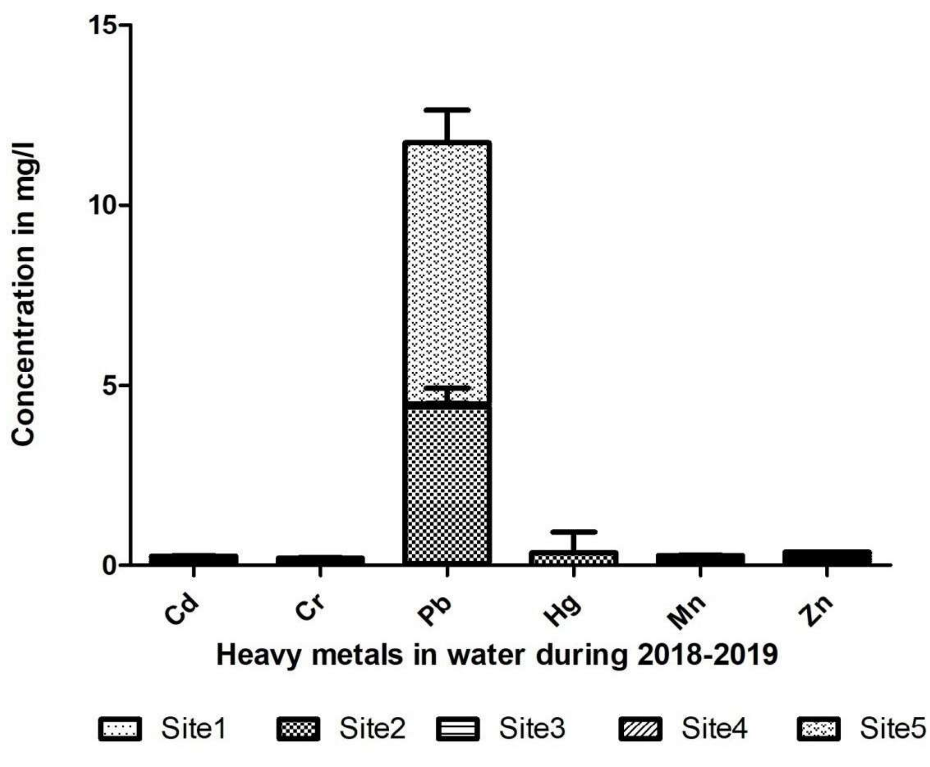

Figure 3: Heavy metals present in water for all five feeding sites of *Ardeola grayii* in 2018-2019

Biochemical Oxygen demand determines the amount of oxygen required for biological oxidation of organic matter with the help of microbial activities. In the present study the value of biochemical oxygen demand ranged between 5.06 to 29.03 mg/L (Table1).

Chemical oxygen demand determines the amount of oxygen required for chemical oxidation of most organic matter and oxidizable inorganic substances with the help of strong chemical oxidant. High COD indicates the presence of all forms of organic matter, both biodegradable and non-biodegradable, and hence the degree of pollution in waters. In the present study the value of COD ranged between 14.69 to 27.82 mg/L which show its high pollution status in S-2 and S-5 sites due to input of domestic drains, dumped plastics, hospitalized wastages, the use of soap and detergents in bathing and washing purposes.

Dissolved oxygen for all five habitats was 4.3 – 7.36 mg/l, DO > 5 mg/l is considered favourable for growth and activity of most aquatic organisms; DO < 3 mg/l is stressful to most aquatic organisms, while DO < 2 mg/l does not support fish life (USEPA 1999). In this study site 2 was having oxygen deficit than others four, site 2 was a very large lake but due to increasing population it was day by day shrinking and filled with garbage. Alkalinity in this study was ranges from 103.03 – 361.7 mg/l. the lake can be categorized as nutrient rich water body and highly productive on the basis of total alkalinity. If any water body have > 20mg/l alkalinity is good for community production, it is also the measure of buffering capacity of water. It is important to assess the alkalinity of water bodies to determine the ability of neutralizing the acidic pollution of water from rainfall of waste water. Total alkalinity is measured on the basis of some components such as bicarbonate, carbonate and hydroxide.

According to Durrani (1993) withdrawal of CO_2 from the bicarbonates for photosynthesis by algae may increase total alkalinity. In this study S-2 and S-3 was having high alkalinity, S-3 was near to temple so by religious activities, people feed fish that causes pond become nutrients rich.

Chloride is found widely distributed in nature in the form of salts of sodium, potassium and calcium. The chloride status in water is indicative of pollution, especially of animal origin. In the present study chloride concentration was found ranging between 22.10 to 59.8 mg/L (table1). Site -2 was having more chloride level due to large amount of organic matter, waste of animals dumped in lake.

Total hardness of water is the measure of alkaline earth elements such as calcium and magnesium in an aquatic body along with other ions such as aluminium, iron, manganese, strontium, zinc, and hydrogen ions. In this study the total hardness of water range was 137.7 to 311.36mg/l in study area. Site 2 had highest 311.36mg/l total hardness in water.

As the content of Ca and Mg in water increases, the content of hardness also shoots up. The average value of calcium hardness for the study period was 29.6-55.4mg/l, whereas the average value for Mg^{2+} recorded was 22.1-60.7 mg/l for the study period. Calcium and magnesium are the dominant cations in an aquatic body. Higher concentration of calcium and magnesium is due to the dissolution of carbonate minerals in water through rainwater mixing, while a lower concentration is due to increased photosynthetic activity of aquatic organisms (Divya 2013).

In 2017-2018 (Table 2) it was observed that, site 2 (7.8 pH) and site 3 (7.23 pH) become slightly alkaline than other three sites. In site 2, DO (4.6mg/l), BOD (8.9 mg/l) lowest and highest COD (41.8mg/l) was observed. Total hardness was maximum in site 2 (308.7mg/l) and site 5 (308.7mg/l), calcium and magnesium was maximum in site 5(56.5 – 60.9mg/l). Alkalinity was observed maximum in site 2 (357.5 mg/l) and site5 (319.8 mg/l).

In 2018-2019, it was observed that (Table 3), site 2 (8.1 pH) and site 5 (8.03pH) was alkaline than other three sites, BOD was minimum in site2 (12.5mg/l), COD maximum in site2 (60.7mg/l), and dissolved oxygen minimum in site 2 (3.8 mg/l) and in site 5 (4.56mg/l). Total hardness of water was maximum in site 2 (333.9 mg/l) and site 5 (351.06 mg/l).

Due to urbanization and industrial processes large quantities of pollutants have continuously been entered into ecosystems. Metals are persistent pollutants that can be biomagnified in the food chains, becoming increasingly dangerous to human and wildlife. This has led to the development of monitoring schemes aimed at directly measuring levels of contaminants in various organisms, and biomonitoring schemes that use indicator species to estimate the levels in other parts of the ecosystem.

Birds, like other organisms, are harmed by heavy metals. For example, metals were affect birds immune system, increase aggressive behaviour, territorial song, and reproductive dysfunction, increased susceptibility to disease and stress and changes in behavioural pattern.

Heavy metals are frequent waste products of industrial and agriculture processes, they enter the food chain via air, water, soil, and biota and their accumulation increases at higher levels of food chain (Burger, 1993). Heavy metals can have harmful effects on development, behaviour and intelligence both in animals and humans (Finkelstein et al., 1998). In this study Cd, Cr, Pb, Hg, Mn and Zn were tested for all five feeding sites of *Ardeola grayii*, it was observed that, in all feeding sites Cr, Mn, and Zn was present in equal proportion than Pb, Cd, and Hg , first three Cr, Mn, and Zn not too much harmful than Pb, Cd, and Hg. In this study for all four year (2019-2019) data were compared by using graph by using mean, SD values and Heavy metals present in water for all five sites of *Ardeola grayii* are given in Figure 1, 2 and 3.

During 2016-2017 lead was present in site 5, site2 followed by site4, mercury present only in site 2.

During 2017-2018, Lead was maximally present in site 5 followed by site2, and mercury present in site 2.

During 2018-2019, level of heavy metals present in all five feeding sites of *Ardeola grayii* was differed for site 2 and site 5, Pb was maximally present in site 5 followed by site2 (Fig. 3), in site Hg also present it indicates site 2 was more contaminated than other four sites.

Table 4: Different pollutants present in feeding habitats and their impact on behaviour of *Ardeola grayii*

Feeding sites	**Pollutants**	**Change in behaviour**
Site-1	Domestic waste disposal site,	Flee behaviour, changes in flight distance
Site-2	Misuse of pond as sewage and domestic waste disposal site , water hyacinth	Flee behaviour, aggression, changes in foraging behaviour
Site-3	Being in proximity to religious complex, people use plastic bags, matchsticks, incense sticks, milk packets, disposable utensils, earthen pots, etc. that are often carried to pond by winds.	Flee behaviour, increase in tolerance level
Site-4	Dumped garbage in pond	Not much contaminated as other sites, normal behaviour seen
Site-5	Tannary waste, automobile wastages	Flee behaviour,

Behaviour is suggested to be a more useful indicator or biomarker than standard assays in laboratory conditions because the harmful effects of pollutants sometimes become only noticeable in natural ecological conditions, such as social stress or infections (Zala and Penn 2004).

In this study it was observed that, *Ardeola grayii* behaviour differed in urban and rural area, because urban areas more polluted than rural area. Site 2 and site 5 was polluted compare to other three sites.

Site 1 was large in size and it inhabitants many water birds, it was not much contaminated, but human disturbance occur there, as a result *Ardeola grayii* seen to frequent change foraging patches.

Anthropogenic activities are some of the major factors in the study area posing significant threat to these wetlands. Water hyacinth has rapidly covered the water surface in Site 2 Lake, thereby, reducing the foraging area for open-water birds. These large, unwanted monotypic stands of water hyacinth could reduce the value of the wetland as potential *Ardeola grayii* habitat (Manral et al. 2013).

Site 3 was polluted due to dumping of wastes materials (such as plastics, polythene bags, chips packets), bathing and offering made in the ponds, during mass bathing by local people are influencing the water quality and avifauna.

Site 4 was in rural area less polluted only fleeing behaviour observed there. Site 5 was also in rural area but it was near to tannery factory so water contaminated.

Ardeola grayii is highly susceptible to continuous anthropogenic pressures in the form of washing clothes, cattle bathing, cattle grazing, and entry of domestic sewage, hunting, fishing, and expansion of crops lands.

Pollution of the environment is one of the terrible ecological disaster to which they are subjected nowadays. Nearly all of the activities of human society have produced unfavourable effects on all living forms in the biosphere. The cause of water pollutants are domestic sewage, detergents, pesticides, chemicals, dead materials and industrial effluents through a variety of processes. Sustaining healthy ecosystems that can save from harm to the organisms existing within them, including humans, necessitates not only ecological planning and management, but also knowledge of how stressors vary in the atmosphere (Burger et al. 2004). More and more it is essential to appreciate the outcome and effect of pollutants to evaluate the health of ecosystems and to bring early warning of alterations in the environment that might specify undesirable effects (Burger, 2002). Wetland bird's populations may provide as sentinel species for natural and anthropogenic pollution problems in the surroundings.

Major foraging grounds of these birds were paddy fields, river banks, ponds, and other water sources, but now these birds are getting adapted to garbage dumps in towns, waste water canals etc. Increase in food source (insects, bugs and worms) may have attracted these birds to garbage.

In this study it was mainly focused on there any impact of water quality on *Ardeola grayii*, it was observed that, it is fish loving bird, and fishes are found in lake, pond, agriculture field, and small ditches, but if there was not life supporting requirement of fishes in water so there was very few or almost fishes absent in that feeding sites as a result *A. grayii* had to skip that site for feeding or shifted foraging behaviour. In urban area due to lack of water bodies or if available choked by garbage and get contaminated so there was no life support for aquatic organism as a result *Ardeola grayii* shifting their dependency on water bodies to garbage or dumped area, where it can get insects, worm, and bugs. But in rural area there was water bodies available so *Ardeola grayii* mostly observed near lake, pond or agriculture field for foraging, there was less disturbance, prey easily available, no polluted water and their number was maximum than urban areas.

In this study it is observed that, water quality affect behaviour of *Ardeola grayii* and their population in any feeding habitats, we should know the importance of water bodies and wading birds for ecosystem and food chain, we should try not to dumped garbage in water bodies, it importance not only for wading birds but also for fishes and other invertebrates which live in water bodies and all those are play important role in maintaining a food chain.

Shrinkage of water surface, decrease in salinity and fishery resources, introduction of invasive fresh water aquatic weeds is the greatest threats to the lake. An overall loss of biodiversity with decline in productivity adversely affecting the livelihood of the community.

CONCLUSION

Ardeola grayii is mostly depend on water body for their life activities, this studies data suggests that, day by day shrinkage of water body and deterioration of water quality increasing so in coming days aquatic birds will come under threatened condition. In this study the feeding habitat of *A. grayii* exhibits low DO, high BOD and COD, total hardness and higher level of concentration of metal level. So it faces threats to the habitats and sites on which they depend for feeding, breeding and resting purposes. In this study it was observed that they shift their habitats but in city area most of the wetlands deteriorating so if wetlands will be not maintained *A. grayii* may comes under threatened category. These birds are indicator of healthy environment, so protection, management and conservation of wetland require so that birds can also save from extinction.

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Conflict of Interest
The author(s) declare(s) that there is no any conflict of interest

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