CHAPTER: 4

ANALYSIS OF TOXIC AND ESSENTIAL ELEMENTS

INTRODUTION:

Large quantities of pollutants are continuously being introduced into the environments of cities as a consequence of anthropogenic activities such as urbanization, vehicular traffic and industrial processes. Among these heavy metals, due to their high potential to enter and accumulate in food chains, are considered as critical contaminants in the environment, (Olojo *et al.,* 2005; Erdoğrul and Erbilir, 2007). The main sources of heavy metal pollution are industries and mining activities (Singh *et al.,* 2007). At low concentrations arsenic, lead, cadmium and chromium are toxic to living organisms while other metals like copper and zinc are the biologically essential metals that become toxic only at very high concentrations (Cohen *et al.,* 2001; Storelli *et al.,* 2006).

Environmental contamination caused due to anthropogenic activities is a relatively novel concept in ecological times. These stressors negatively affect individuals and entire ecosystems via air and water around the world, even in the areas relatively free of their production (Iwata *et al.,* 1993). However, in the past few decades, more efforts have been made to discuss the monitoring of health of the ecosystem. These discussions concentrate on presence of essential, non essential elements and other pollutants in the environment that cause great risk to all living biota. These elements are frequent waste products of industrial activities and their emission contaminate surrounding environment (Eeva and Lehikoinen, 2000).

Increasing levels of toxic and essential elements in air, soil as well as water are of growing concern for the biota on earth including human being. There are hundreds of sources for these elements like pollution, combustion of coal and natural gases, paper and chloroalkali industries, *etc.* through which these metals end up and accumulate in environment. Their excessive persistence in the environment or their bioaccumulation and biomagnification in food chains are the potential threats leading to undesirable effects in the biota (Zhuang *et al.*, 2009; Dżugan *et al.*, 2012). A control of accumulation of these substances in an ecosystem is of great value in context to global environmental pollution. Use of bioindicators in monitoring such pollutants is often easier compared to monitoring abiotic samples (Lebedeva, 1997). Among the bioindicators birds are traditional subjects for monitoring polluted ecosystem, especially in territories adjacent to stationary sources of pollution. The studies on birds with reference to accumulation of heavy metals are extensive and increasing (Dmowski, 1999).

Birds occupy wide range of tropic levels in different food chain. Due to this compared to other species it is very easy to monitor them (Burger *et al.,* 1999; Eens *et al.,* 1999). Among them species that are at the apex of the food web are more commonly used as bio-indicators to evaluate the presence of persistent contaminants. As a result of escalating generation of evidences that bird populations are particularly sensitive to the changes produced by men in the environment, potential use of birds as indicator for environmental pollution has been widely recognized. Most of these species are long-lived, hence pollutant burden in their bodies get integrated in a complex way over the time. Thus, birds are useful biomonitors for studying bioaccumulation. They are visible, sensitive to environmental changes and are at the pinnacle of the food chain. Raptors are amongst the most intensively studied bird species in biomonitoring investigations due to their top position in the food chains and the spatial integration of contaminant levels in their extended home ranges (Altmeyer *et al.*, 1991, Esselink *et al.,* 1995, Garcia- Fernandez *et al.,* 1997, Pain *et al.,* 2007).

Their ecology, physiology and behaviour have been well studied and they are of interest to the public (Burger, 1993). Most studies have been conducted on internal tissues, but the number of studies making use of non-destructive methods, like measuring the concentrations of metals in feathers, faeces and eggs have increased over the years. Especially, feathers are considered as valuable tool to monitor exposure to heavy metals. However, many carnivorous birds are foraging over a wide geographical area; due to this it is also very difficult to detect source and site for their toxic ingestion. The effects of heavy metal concentration in the different species have been studies by researchers like Burger (1993), Furness (1993), *etc.* The environmental toxicant can bind to the proteinmolecules in the feather during the short period of feather growth when the feather is connected with the bloodstream in pulp of calamus (Furness, 1993). Compared to blood and other tissues it is easier to detect and quantify heavy metals in feathers (Cahil *et al.*, 1998; Tom *et al.*, 2002).

The Black Kite (*Milvus migrans govinda*) is a common diurnal scavenger distributed throughout the Paleartic region (Bijlsma, 1997) and also a very common scavenger around towns in India (Galushin, 1971; Ali, 1979; Navroji, 2007). In the present study, while studying ecology of kites in urban area an attempt is made to quantify and assess the impact of various elements from different tissue of Black Kite (*Milvus migrans govinda*) collected from one of the most polluted city Ahmedabad, Gujarat, India.

MATERIALS AND METHODS:

The samples of tissue and feather of kites were collected from Ahemdabad (23.03° N 72.58° E) about 90 Km. North of Vadodara. Gujarat is a cultural state where all festivals are celebrated. One of the most celebrated festival of Gujarat is "Uttraryan-The Kite flying festival" celebrated on 14th and 15^{th} January every year. During this festival thousand of paper kites are flown in the sky with sharp threads and there is an impulsive competition among kite flyers to cut each other's paper kites. Due to the sharp threads hundreds of birds are injured. These injured birds are brought to bird rescue centres and are treated by several NGOs and Forest Department, Government of Gujarat for recovery. However, some heavily injured birds succumb to the injury. From these dead Black Kites *Milvus migrans govinda* (Plate 9) from Ahmedabad were used for quantification of heavy metals.

Sample collection:-

Ahmedabad being a Metro city with highest human population in Gujarat has larger population of kites too and hence the number of kites getting injured and succumbing to death is also higher.

After taking Permission from Ministry of Environment and Forest Government of India (MOEF), New Delhi (vide letter no.WLP/28/C/396-398 and F.No.1-4/2007 WL-I) for collection of various tissue from dead birds the samples were collected. For heavy metal analysis, liver, kidney, muscles and primary feathers were taken. Each dead Kite was weighed by digital weighing balance, dissected open and tissues were collected and stored in dry ice and transferred to laboratory and stored at -20^0 C till further analysis. Before analysis the tissues were washed alternately with deionized water (Mili-Q) and acetone and then exposed to 60 ^{0}C in dry oven for 24 h to determine dry weight. The dry tissues were digested in a 1:1 mixture of Nitric acid (Qualigen) (70%) and Hydrogen Peroxide (Merck) (30%). For analysing toxic and essential elements in feathers, first three primaries were taken (Plate 9); their calamus was removed and processed in similar way. The digestion was completed with heat destruction procedure described by Blust *et al.* (1988). All the samples were diluted by adding 4 ml deionized water (Milli Q) and stored at -20 ^{0}C until analysed. Analysis was carried out by Inductively Coupled Plasma Atomic Mass Spectrometer (ICPMS) method described by Swaileh and Sansur (2006) at Inductively Coupled Plasma (ICP) Lab, Pawai, Mumbai.

Statistical analysis:

Statistical evaluation of the data was carried out by using statistical software package SPSS 7.5 for Spearman correlation coefficient while Prism 3 for one way ANOVA. To find out relationship between body weights and concentrations of different metals in different organs of male and female, Pearson coefficient was carried out. Concentration of essential and toxic metal in different tissue like Feathers, Kidney, Liver and Muscles were compared by using One way analysis of variance (ANOVA). To reveal the overall variation in the concentration of each element the coefficient of variation (CV) was calculated. Data values are expressed as mean ± SE and test statistics were considered significant at $P< 0.05$, $P< 0.01$, $P<0.0001$.

RESULTS:

Concentration of toxic elements in Feathers, Liver, Kidney and Muscles of *Milvus migrans govinda*: (Cd, Hg, Ni, Pb, Cr) (Table: 1, Fig: 1)

The mean concentration of toxic metals, cadmium, mercury, nickel, lead and chromium in feathers are 0.05 ± 0.01, 0.03 ± 0.03, 0.1 ± 0.01, 0.4 ± 0.01, 0.25 ± 0.04 µg/g dw respectively. Among other tissues concentration of Cadmium is highest in Liver 2.0 ± 0.01µg/g dw, followed by kidney 0.8 ± 0.03 and muscles 0.2 ± 0.1 µg/g dw. Similarly concentration of Mercury is also high in liver 0.5 ± 0.01 µg/g dw, followed by kidney 0.2 ± 0.02 µg/g dw and low in muscles 0.05 ± 0.01 µg/g dw. The concentration of Nickel is 0.3 ± 0.01, 0.21 ± 0.01 and 0.01 ± 0.01 µg/g dw respectively, while those for Lead and Chromium are also highest 1.0 ± 0.01 and 0.8 ± 0.01 in liver, followed by 0.13 ± 0.01 µg/g dw and 0.15 ± 0.02 µg/g dw in kidney and lowest 0.03 ± 0.01 and

0.04 ± 0.02 µg/g dw in muscles. Accumulation of metals (in µg/g dw) can be summarised as follows: Feathers Pb > Cr > Ni > Cd > Hg, Liver Cd > Pb > Cr > Hg > Ni, Kidney Cd > Hg ≈ Ni > Cr ≈ Pb, Muscles Cd > Hg > Cr > Pb > Ni. The difference in the concentration of toxic elements in tissues studied are highly significant at $P < 0.0001$ with Cadmium- $F_{3,21}$ 64.99, Mercury- $F_{3,21}$ 27.38, Nickel- $F_{3,21}$ 72.21, Lead- $F_{3,21}$ 247.8 and Chromium- $F_{3,21}$ 76.52.

Concentration of essential elements in Feathers, Liver, Kidney and Muscles of *Milvus migrans govinda*: (Zn, Cu, Co) (Table:1, Fig:1)

The mean concentration of essential elements Zinc, Copper and Cobalt in feathers are 26 ± 0.4, 4.0 ± 0.5 and 0.05 ± 0.01 µg/g dw respectively. Amongst the tissues also the concentrations of Zinc is highest of all the essential elements and are 85 ± 0.8, 32 ± 0.9 and 2.0 ± 0.05 µg/g dw for liver, kidney and muscles respectively. The concentration of Cobalt is minimum amongst all the tissues with 2.0 ± 0.01, 1.3 ± 0.3 and 2.0 ± 0.3 µg/g dw in the three tissues respectively while the concentration of copper is 15 ± 0.09, 10 ± 0.7 and 13 ± 0.52 µg/g dw respectively. For essential elements also differences in accumulation of elements in tissue are highly significant at $P < 0.0001$ with Zinc- $F_{3,21}$ 255.9, Copper- $F_{3,21}$ 12.02 and Cobalt- $F_{3,21}$ 34.9. Mean concentration of essential elements can be summarise for feather, liver and kidney as Zn > Cu > Co while for muscles as Cu > Zn ≈ Co.

Correlation between body weight and accumulation of heavy metal in different tissues:

As given in Table: 2, Pearson correlation was employed to examine the correlation between body weight and toxic and essential metal accumulation in different tissues. The body weight of Black kites ranged between 300 to 550 gms. with mean body weight 473.8 ± 13.55. Body weight correlated nonsignificantly either positively or negatively. Both the trends are seen in liver (Cd, Pb, Cu - positive and other elements Cr, Hg, Ni, Zn Co - negative). In kidney it is mainly negative (Cd, Hg, Pb, Cr, Cu, Co,) while only Ni and Zn showed positive correlation. In muscles and feather the correlation with body weight are mainly positive (Hg, Pb, Cr, Zn, Cu) while other elements (Cd, Ni, Co) showed negative correlations.

Comparison of heavy metals in Male and Female Black Kites:

For the comparison of heavy metal concentrations in various tissues of male and female Kites t test is employed. The Results are given in Table: 3. As seen in the table no significant difference are observed in concentration of all elements studied in all tissue of male and female Black kites.

Correlation of concentration of toxic and essential elements in various tissues of male and female Kites with body weight:

Fig (2) shows correlation between body weight and concentration of toxic and essential elements in various tissues of male and female Kites *(Milvus migrans govinda)*. When toxic elements in liver of male and female individuals are considered no significant increase with the increase in body weight in the concentration of all toxic elements are noted except for mercury in male which shows significant increase at $P<0.05$ with body weight. While in female liver non significant decline is noted only for Pb and Cr with increase in body weight. However, in kidney of male and female individuals all toxic elements showed a non significant increase with body weight except chromium concentration. Chromium concentrations are negatively and nonsignificantly correlated with body weight of male birds while positively and significantly at $P<0.05$ with body weight of female birds. For muscles, though the concentration of all toxic elements is very low they showed nonsignificant increase with body weight in both the sexes. Feathers showed varied relationship. In male birds non significant positive correlation for all elements except Ni is noted. In feathers from female kites concentration of Cd, Ni and Cr showed nonsignificant increase with the body weight whereas Hg showed significant increase ($P<0.05$) while Chromium showed nonsignificant decline with body weight.

Among essential elements all showed nonsignificantly increase ($P<0.05$) with body weight in liver and kidney of both the sexes. In muscle negative correlation with body weight is noted only for copper in male while for feathers negative correlation is established in Zinc concentrations in both the sexes.

Coefficient of variation of all elements in different tissues:

All toxic and essential elements in tissues studied are compared with the help of coefficient of variation too (Table:5, Fig:3). In the liver, Mercury concentration showed highest coefficient of variation while Cadmium and Zinc showed lowest CV compared to all other elements. In the kidney with Mercury, Copper also showed highest coefficient of variance while nickel showed lowest variations. In muscles and feather, it is Cadmium concentrations which has highest CV while Copper and Nickel respectively showed lowest CV.

Table:1 **Concentration (in µg/g dw) of toxic and essential elements in Feathers, Liver, Kidney and Muscles of *Milvus migrans govinda*: (Cd, Hg, Ni, Pb, Cr)**

	Feather	**Liver**	**Kidney**	**Muscle**	**F value (***)**
Cd	0.05±0.01	2.0 ± 0.01	0.8 ± 0.03	0.2 ± 0.1	64.99
Hg	0.03 ± 0.03	0.5 ± 0.01	0.2 ± 0.02	0.05 ± 0.01	27.38
Ni	0.1 ± 0.01	0.3 ± 0.01	0.21 ± 0.01	0.01 ± 0.01	72.21
Pb	0.4 ± 0.01	1.0 ± 0.01	0.13 ± 0.01	0.03 ± 0.01	247.8
Cr	0.25± 0.04	0.8 ± 0.01	0.15 ± 0.02	0.04 ± 0.02	76.52
Zn	26 ± 0.4	85 ± 0.8	32 ± 0.9	2.0 ±0.5	255.9
Cu	4±0.05	15±0.09	10±0.7	13±0.52	34.9
Co	0.05±0.01	2.0±0.01	1.3±0.3	2.0±0.3	12.02

Table: 2 Overall Correlation of various toxic and essential elements in tissue with body weight (Pearson correlation).

Metals	**Feather**	**Liver**	**Kidney**	**Muscle**
Cd	-0.06	0.77	-0.05	-0.06
Hg	0.16	-0.04	-0.38	0.17
Ni	-0.17	-0.001	0.07	-0.12
Pb	0.16	0.33	-0.06	0.16
Cr	0.61	-0.56	-0.75	0.67
Zn	0.08	-0.06	0.11	0.09
Cu	0.52	0.26	-0.04	0.80
Co	-0.03	-0.05	-0.03	-0.004

Table: 3 A comparison of Toxic and essential elements in various tissue in Male and female *Milvus migrans govinda* (t test)

	Feather		Liver		Kidney		Muscle	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Cd	0.01±0.03	0.05±0.01	0.92 ± 0.07	0.86 ± 0.08	0.92 ±0.09	0.92±0.07	0.48±0.08	0.43±0.10
Hg	0.05±0.01	0.04±0.01	0.51 ± 0.07	0.47 ± 0.06	0.22 ± 0.02	0.20 ±0.02	0.1 ±0.01	0.08±0.07
Ni	0.07±0.01	0.09±0.01	0.3 ± 0.02	0.29 ± 0.03	0.24 ±0.01	0.24 ±0.01	0.03 ±0.08	0.02 ±0.05
Pb	0.41±0.08	0.26±0.05	1.14 ± 0.06	1.13 ± 0.08	0.16 ±0.01	0.15 ±0.01	0.05±0.01	0.06±0.1
Cr	0.22±0.04	0.25±0.06	0.68 ± 0.06	0.61 ± 0.04	0.19 ± 0.006	0.17 ±0.01	0.06±0.007	0.05±0.005
Zn	27.28±4.99	21.83±4.45	81.0±3.27	79.33±2.72	37.5 ±2.79	38.3±1.90	4.88±1.02	4.50±1.057
Cu	1.92± 0.38	3.23±0.477	15.83±1.62	15.67±1.52	13.92±1.55	13.3±1.54	14.83±0.90	14.17±0.70
Co	0.019±0.06	0.049±0.01	1.93±0.133	1.94 ±0.1	1.57±0.11	1.41±0.1	3.45±0.45	3.0±0.365

All p values are Non significant (NS)

Table: 4 Correlation of concentration of toxic and essential elements in various tissues of male and female kites *(Milvus migrans govinda)* with their body weight.

	Liver		Kidney		Muscle		Feather	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Cd	0.71	0.41	0.54	0.43	0.65	0.17	0.13	0.38
Hg	0.81*	0.33	0.58	0.44	0.79	0.44	0.63	0.87*
Ni	0.78	0.49	0.52	0.47	0.7	0.7	-0.6	0.47
Pb	0.65	-0.57	0.03	0.23	0.8	0.24	0.01	0.39
Cr	0.64	-0.1	-0.05	0.89*	0.35	0.06	0.62	-0.23
Zn	0.82	0.91	0.44	0.4	0.84	0.49	-0.52	-0.02
Cu	0.81	0.8	0.47	0.23	-0.63	0.14	0.46	0.02
Co	0.75	0.56	0.74	0.05	0.54	0.59	0.50	0.11

*P < 0.05

Table: 5 Coefficient of variance (CV) of all elements in different tissues of *Milvus migrans govinda*.

Elements	Feather	Liver	Kidney	Muscle
Cd	101.75	9.03	18.85	56.81
Hg	55.19	30.27	27.96	21.30
Ni	45.84	20.10	13.02	55.82
Pb	54.31	13.05	21.92	45.82
Cr	51.35	22.35	14.62	24.52
Zn	46.47	9.44	14.86	54.84
Cu	47.26	23.91	27.01	11.59
Co	83.86	16.10	17.76	28.42

Fig:1 Concentration of toxic and essential elements in Feather Liver, Kidney and Muscle of *Milvus migrans govinda*:

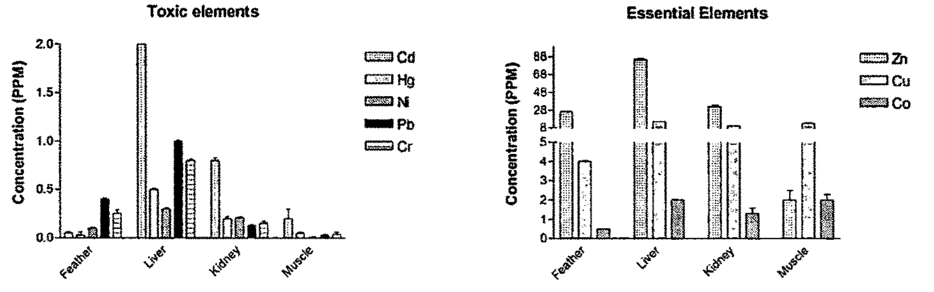
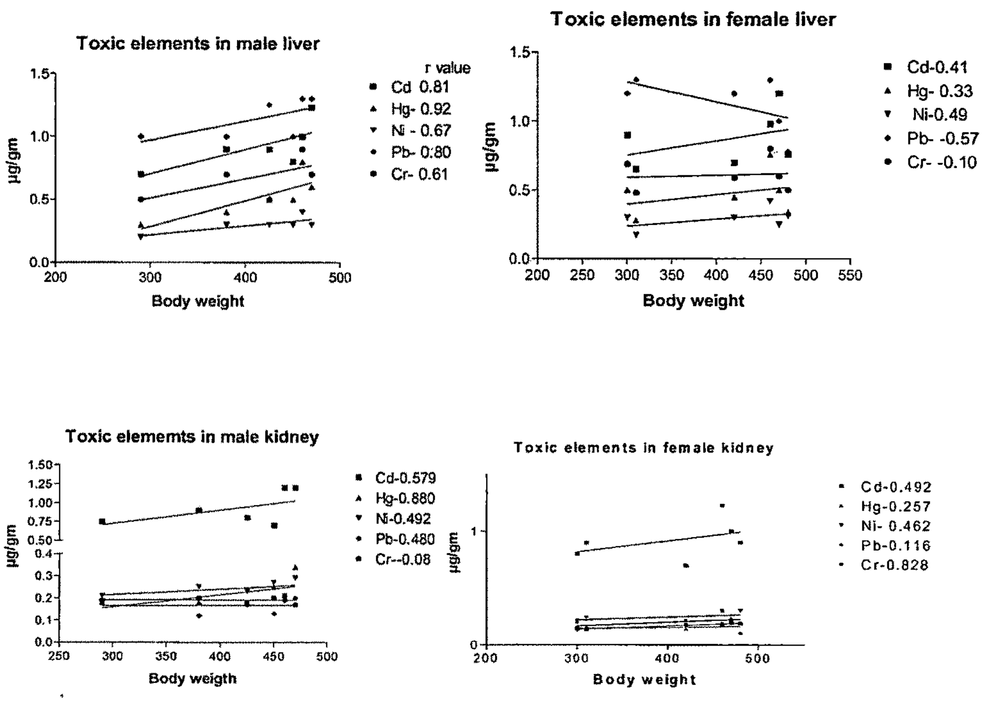
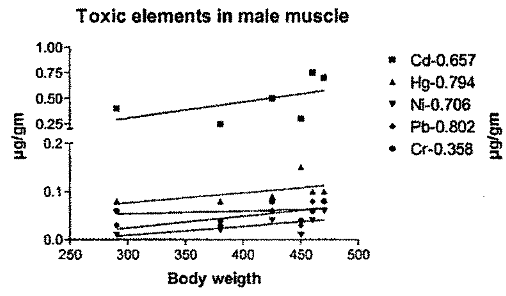
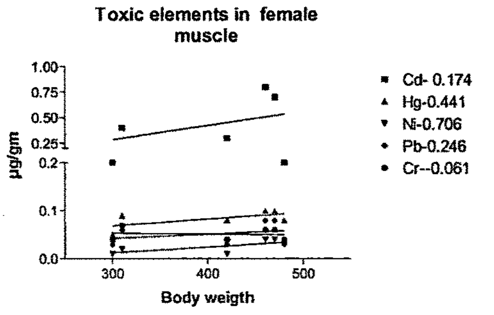
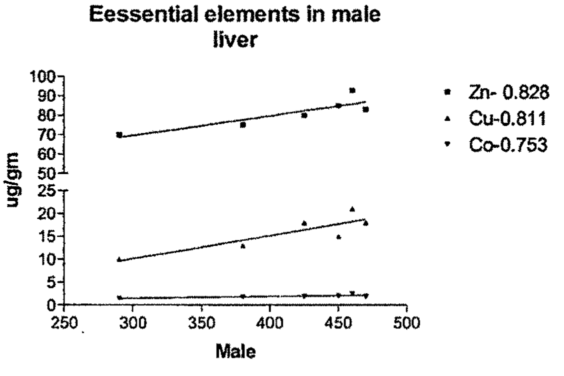


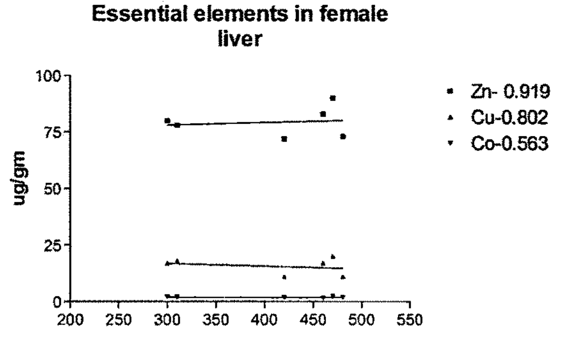
Fig: 2 **Correlation of tissue concentration of male and female Black Kites with Body weight.**

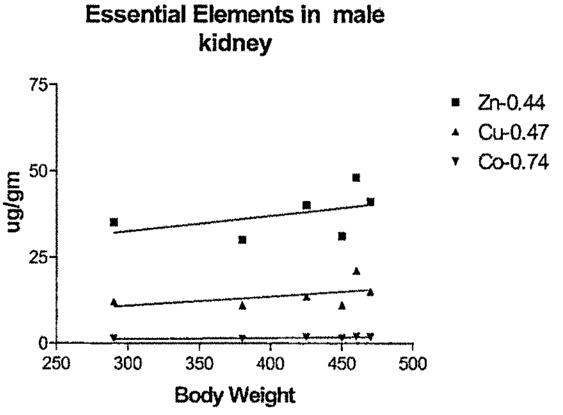


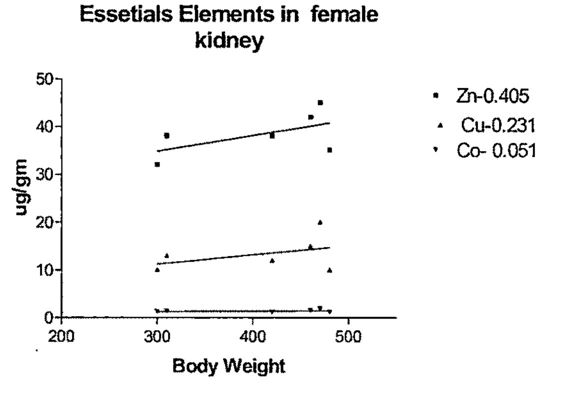

Toxic elements in male muscle
µg/gm
Body weigth
Cd-0.657
Hg-0.794
Ni-0.706
Pb-0.802
Cr-0.358

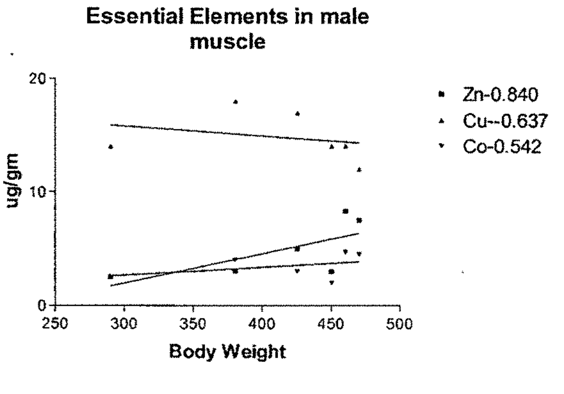

Toxic elements in female muscle
µg/gm
Body weigth
Cd- 0.174
Hg-0.441
Ni-0.706
Pb-0.246
Cr--0.061


Eessential elements in male liver
ug/gm
Male
Zn- 0.828
Cu-0.811
Co-0.753


Essential elements in female liver
ug/gm
Zn- 0.919
Cu-0.802
Co-0.563


Essential Elements in male kidney
ug/gm
Body Weight
Zn-0.44
Cu-0.47
Co-0.74


Essetials Elements in female kidney
ug/gm
Body Weight
Zn-0.405
Cu-0.231
Co- 0.051


Essential Elements in male muscle
ug/gm
Body Weight
Zn-0.840
Cu--0.637
Co-0.542

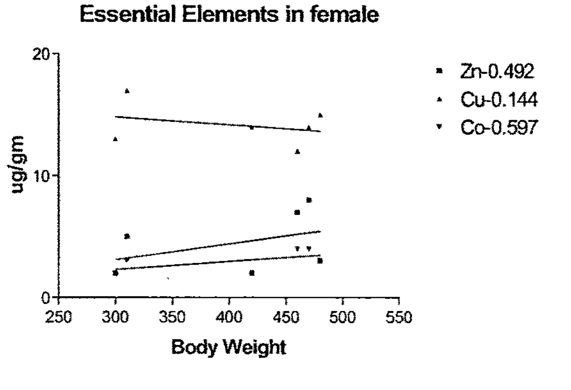
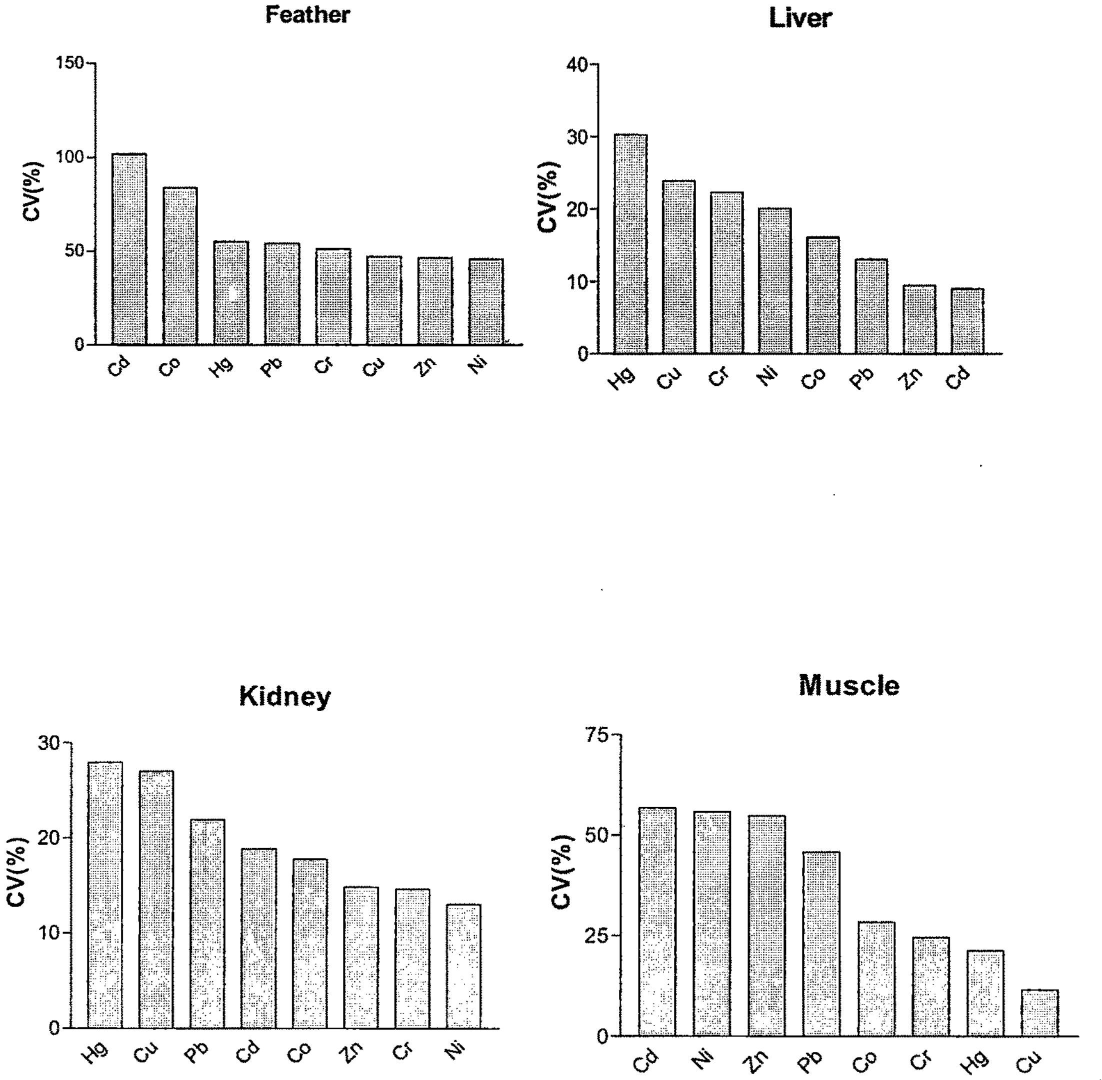

Essential Elements in female
ug/gm
Body Weight
Zn-0.492
Cu-0.144
Co-0.597

Fig 3: Coefficient of variance of all elements in different tissue of Black Kite



DISCUSSION:

Avian survivorship in urban areas is influenced by risk of environmental toxicants, collision with man-made objects, changes in the predator assemblage, food supply and diseases (Chace and Walsh, 2006). Among the environmental toxicant metal and elements form major component. Many studies reported on concentration of heavy metals in different groups like Birds and fishes (Licata *et al.,* 2010; Mansouri *et al.*, 2012; Binkowski *et al.,* 2013, Begum and Sehrin, 2013, Rajkowsa and protasowicki, 2013, *etc.*). Birds are used intensively for the past 30 years as a biomonitoring tools (Burger, 1993). Among eight elements Studied Cadmium is the toxic element which is showing maximum accumulation among the elements studied in the three tissues. However, its CV was maximum >100% for feathers followed by kidney and liver. Cadmium is known to be nephrotoxic (Gancia- Fernandez *et al.*, 1997; Kalisinska *et al.,* 2004). Studies of Binkowski *et al.,* (2013) show highest concentration of Cadmium in kidney of Waterfowls *i.e.* Coots and Mallards indicating similar efficiency of detoxification process. However, in terrestrial raptor *Milvus migrans govinda* highest cadmium concentration are noted in liver. A non significant but positive correlation of hepatic cadmium levels while negatively correlated with kidney, muscle and feather concentration with respect to body weight indicates a possibility of difference in efficiency of detoxification process in water dependent and terrestrial species which needs to be further explored. As high concentration of Cadmium also occurs in liver it has been reported that Liver and Kidney together may accumulate about 75% of Cadmium in an organism (Gunn and Gould, 1957, Nordburg *et al.,* 2007) as it is also true in present studies. Further, as per various studies (Nicholson, 1981, Thompson, 1990) in present study also Cadmium levels in muscles and feathers are low. Singh and Srivastav (2007) state that the tissue concentration of metal content is strictly influenced by availability to animals while those in feathers to the share deposited in growing feathers through blood (Furness *et al.*, 1990).

Mercury is one of the most important heavy metal toxicant in the environment originating from both natural cycling in the biosphere as well as anthropogenic activities like industrial emissions, burning of fossil fuels *etc*. As top predators as well as urban exploiters, Black Kites- *Milvus migrans govinda* is likely to accumulate the same in its body. Among the three tissues studied and feathers, maximum Hg concentration occurred in liver, followed by kidney and low in Muscles and feathers. Mercury contents in feathers are the indications of mercury contents in the diet of an individual (Furness *et al.,* 1990). Top predators are known to accumulate high mercury contents in their feather too. Though, Black

Kite is a raptor it mainly scavenges on the poultry left over (Rathod and Padate, 2004, Chapter:III). Hence, the accumulation of the same in feathers can be low. However, in Black kite accumulation of Hg in liver is highest followed by Kidney, indicating presence of the same in the terrestrial urban environment of Ahmedabad. This also indicates that Liver and Kidney of Black kite (*Milvus migrans govinda*) are able to handle the low concentrations of this heavy metal in the environment. However as far as CV is concerned feathers showed 55% variability compared to other tissues which showed CV between 20 to 30 % indicating that in these individuals that are exposed to Hg load accumulation of Hg increases in feather. The liver is preferred organ for metal accumulation. When mercury concentration is correlated with body weight positive correlations are obtained for muscles as well as feathers while negative correlations with liver and kidney. *Milvus migrans govinda* shows higher accumulation of Chromium too in both tissues. With significantly low accumulation in other tissue indicating that liver is able to handle this load of mercury too. However, among other three tissues accumulation of chromium is higher in feathers with 50% CV against 15 to 25% CV of other tissues indicating irregular ingestion of this metal in food by *M. m. govinda*.

Nickel, a ubiquitous metal in the biosphere is introduced in the environment from natural and human sources. It is circulated via chemical as well as physical processes and transported across living organism via biological mechanisms (Sevin, 1980; WHO, 1991). Though useful for normal growth of many microorganisms, plants and several species of vertebrates WHO classify this metal as carcinogenic for humans. Hence, nickel which persist in the environment and has affinity for bioaccumulation is considered as toxic metal (Bubb and Lester, 1996). The data on toxic influence of Nickel on birds is more limited than mammals. Outridge and Scheuhammer (1993) report higher accumulation of nickel in mammals and birds form Ni polluted environment with occasionally kidney showing higher levels than liver. According to these authors higher doses of nickel 300 to 800 μg/g in diet of chicks produces variable effects including death whereas still higher dose in adult show no evidences of systemic or reproductive toxicity. Hence, tissue concentrations of nickel are not reliable indicator of potential toxicity. DeForest *et al.,* (2012) states that the nickel toxicity is likely to occur in animals ingesting food that includes soil that are specifically higher in earthworms. However, concentrations higher than 10 μg/g dw of kidney and 3 μg/g in liver are considered to lead to no toxicity. Aquatic food chains with macrophytes and aquatic organisms like piscivores, or terrestrial food chain like soil earthworm and worm eating mammals, are at risk of secondary poisoning (Technical Guidance Document, 2003). On the basis of very limited data available for correlation of bioavailability of the diet born fraction of nickel in birds, the concentration of nickel in various tissues of Black Kites may be considered as below the toxic levels. Further, Kites do not fall in any of the food chains which are believed to lead to bioaccumulation of this metal leading to its toxic effects. Birds all over the world usually show low accumulation of nickel (Kozulin and Pavluschick, 1993; Hui, 1998; Hui *et al.,* 1998). However, accumulation of nickel is positively correlated with body weight only in kidney while in other three tissue it is negatively correlated. As, Nickel concentration is higher in feathers and muscle with 45- 56% CV against 10 to 20% CV of other tissues it indicates irregular ingestion of this metal in food of *M. m. govinda.*

Lead toxicity in birds is frequently associated with ingestion of lead shots, mistakenly consumed as food particles or grit or from dead prey which has been shot by lead pellets. These shots remaining in muscular gizzard can lead to Lead toxicity (Sanderson and Bellarose, 1986; Pain, 1992; 1996, Scheuhammer and Norris, 1996). Hunting of 1.4 to 2.6 million water birds annually has been estimated by U.S. Fish and Wildlife Services (1986). The status of "Critically Endangered" Callifornia Condor *Gymnogyps califernianus* has been associated with Lead poisoning from such ingested bullets (Wiemeyer *et al.,* 1988; Birdlife international, 2007). This has also been reported to be major obstacle to the reintroduction programs for this species (Meretsky *et al.*, 2000). Several other species are also moved to critical and near threatened categories due to lead poisoning. However, as Kites mainly feed on poultry leftover (Rathod and Padate, 2004; Chapter III), there is rare possibility of heavy metal load of lead to this species. Nevertheless, here liver showed maximum concentration followed by feathers, kidney and Muscles. It showed positive increase in liver and muscles as body weight increased. While among other two tissues accumulation of lead is higher in feathers with >50% CV. Clark and Scheuhammer (2003) considered <6 µg/g dw lead in Liver and Kidney of birds as back ground concentration while >6 µg/gm associated with exposure to lead and >20 µg/gm liver and >30 µg/gm as toxic. Hence, the concentration in present study indicates much lower exposure of Black Kites of lead in city of Ahmedabad. The muscles in general are considered to have weak accumulation potential (Erabgrual and Erbilir, 2007; Bervoers and Blast, 2003).

Having multiple valance chromium has dual character. Chromium III plays key role in the metabolism of animals (e.g. formation of glucose tolerance factor), while its hexavalent form Cr VI is related to occupational health hazard (Jeejeebhoy *et al.*, 1977; Gad, 1989). Hence, Chromium is known to be essential for human as well as animals (Schwartz and Mertz, 1995). Though homeostatic mechanism regulates chromium uptake as per nutritional requirements of body an increase in chromium concentration is observed when mechanisms are saturated or are less effective in controlling uptake. In such cases Chromium intoxication may be observed. Since the first reports of Cancer in early 1990s, there have been several studies on chromium levels in environment and biota including birds (Mansouri, *et al.*, 2012; Toghyani, 2012; Binkowski, *et al.*, 2013). However, no biomagnifications of chromium has been reported in food chain probably due to ready ability of Cr VI to get converted to Cr III. This ability is likely to protect the higher organisms from the effects of low level of exposure (Eisler, 1986). In present study, among the tissues studied though highest Chromium levels are in liver and lowest in muscles, the overall concentration is low. As said earlier in urban areas of Gujarat, Black Kites get plenty of Poultry left over (Skin with feather, Rathod and Padate, 2004; Chapter:III) where exposure to chromium is expected to be nil. Hence, it can be said that whatever Chromium occurs in tissues of Black Kites has other sources and have not crossed the critical levels to consider as harmful to kites. However, as body weight increases, consumption of food also increases and the accumulation of metal is significant as is evinced by increase in Chromium concentration with increase in body weight in muscles and feathers. Metal level in feather may change and increase with age due to exogenous contamination.

Zinc an essential trace element is having varied functions in body (Welsh *et al.,* 1994). However it is also believed to be harmful to organisms at higher concentrations (Sandstead and Au, 2007). In human Zn accumulation is considered as less toxic. The interaction of Zinc and Copper is considered to be mutually antagonist and hence probably in livestock Zinc supply is raised as a protective measure to reduce Copper toxicity. It also plays important role against Cadmium toxicity (Jacob *et al.,* 1987). Animal flesh is the best source of readily bio available Zinc (Soriano-Santos and Guerrero-Legarreta, 2010) with red meat being richest. Among birds the aviary species are likely to exhibit Zn poisoning due to galvanized irons (Cage wire, cage clips and bird toys). However, toxicity associated to Zn coated food containers has been reported in humans but not in birds (Brown *et al.,* 1964). Toxic level of Zn in liver of different aviary species of psittacine species is reported to range from 95 to 759 µg/g dw (Puschner *et al.,* 1999). Though, higher levels of Zn are recorded compared to other heavy as well as essential elements in the tissue of Black Kites, in present study these levels are nowhere near toxic concentrations. Among essential elements Zinc concentration in tissue studied except liver, increased positively with body weight. While in other three tissues muscles and feathers this increase was with 45-55% CV against 5 to 20 % CV of Liver and Kidney of *M. m. govinda*. Black Kite *Milvus migrans govinda* is neither aviary bird nor it feeds on red meet (it gets plenty of poultry left overs *i.e.* white meat in urban area). Hence, it can be said that the possibility of any zinc toxicity in Black Kite is remote in present environmental conditions.

Copper, the naturally occurring metal in variety of foods like nuts, meat and grains, is one of the important micro nutrient essential to human health. However, being ubiquitous in the environment, it is highly toxic to many freshwater aquatic species including algae and fishes. Terrestrial fauna is exposed to acute and chronic copper toxicity risk due to agricultural pesticides. Because of its role as essential trace element there is uncertainty in establishing its risk to birds and mammals. Many of these have the ability to handle excess copper by storing it in liver and bone marrow. Hence copper deficiency as well as toxicity risk are rare. In accordance to the studies of Szymczyk and Zalewski (2003) and Binkowski *et al* (2013) the copper concentrations in tissues of Black Kites in present study are found to be lower with low concentrations in muscles compared to liver as reported by former ornithologist. As body weight increased concentration of Copper also increased in muscles, followed by feathers and liver. The concentration of Copper in tissues especially liver is regulated below 50 μg/g dw by homeostatic control (Pyle *et al.,* 2005).

Cobalt, a compound of Vitamin B_{12}, in a minute quantity is an accessory element for life mainly in production of erythrocytes and prevention of anaemia. At higher levels of exposure it has been shown to produce mutagenic and carcinogenic effects which may be similar to nickel. Its concentration in body is highly regulated with over 80 % accumulated in the skeletal system. Mean range of Cobalt in different species of sea birds has been shown to be between 0.048 to 0.11 μg/g dw with lowest coefficient of variance (Szefer *et al.,* 1993; Kim *et al.,* 1998). Further, accumulation of cobalt in eggs of tern has shown no change in levels for eleven years (Burger and Gochfeld, 1988). However, in the present study cobalt levels in tissues are found to be much higher from 0.05 in feathers to 2.0 μg/g dw in liver and muscles. However, compared to three tissues accumulation of cobalt is elevated in feathers with >80% CV against 15 to 30% CV of other tissues of *M. m. govinda.* This needs further investigation with reference to its role in terrestrial raptors in one of the highly polluted urban area of India.

Gender Difference:

In present study the accumulation of heavy metals showed no gender difference as is demonstrated in the Red- Billed Gulls (*Larus novaehollandiae scopulinus*) (Furness *et al.,* 1999), Great tit (*Parus major*) and Blue tit (*Parus caeruleus*) (Dauwe *et al.*, 2002). However, in present study only Mercury and Chromium concentrations showed significant increase in either of sexes in relation to body weight while, rest of elements showed nonsignificant increase or decrease. Burger and Gochfeld (1997) found significant differences in levels of several metals between male and female ducks, although mercury levels were the same in both the sexes. Hutton (1981) found differences in levels of Zinc and Cadmium between male and female Oystercatchers *Haematopus ostralegus* while Heinz (1976) has reported higher Mercury levels in the livers of males than in those of females in Mallards, after the egg-laying period.

Few studies have examined the effects of gender on the accumulation of heavy metals in feathers and other tissues (Burger, 1995; Gochfeld *et al.*, 1996). In feathers of Bonaparte's Gulls, Braune and Gaskin (1987) found significantly lower levels of Mercury in primary feathers of female when compared with primaries of male birds while in present study no gender difference is found in accumulation of essential and toxic metals in feathers of *Milvus migrans govinda*.

Biological monitoring provides direct qualitative and quantitative assessment of exposure of a group of persons or individual to noxious agents present in the environment (Mehra, 2010). Advantages of using biomonitors are recently acknowledged. To identify pollution in a larger area Bio-indicators that reflect a coarser spatial scale should be used. Birds use different sources of food and water in a relatively large area and thus the levels of trace elements in bird's organs and feathers are expected to reveal the levels of toxic elements in their entire home range. In the present study it is revealed that urbanization in Central Gujarat has very little impact on accumulation of heavy metals in various tissues of Black Kites and hence the lower concentrations of these metals in tissue of Black Kite (*M. m. govinda*) which mainly feeds on poultry leftovers which are expected not to be exposed to heavy metals as poultry is one of the major food item in the diet of urbanized human population. The Black Kite has exploited this food very well and hence is thriving in urban areas due to plenty of food supply, thermals to soar at high levels in the sky with little exposure to polluted diet and air at lower levels.

Plate 9 Dead Black Kite (*Milvus migrans govinda*) during Kite flying day due to sharp thread at Ahemdabad.

Position of three primaries of Black Kite (*Milvus migrans govinda*)