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QUANTIFICATION AND ASSESSMENT OF VARIOUS ENVIRONMENTAL TOXICANTS FROM FEATHER OF BLACK KITE (*MILVUS MIGRANS GOVINDA*): A PRELIMINARY STUDY

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ABSTRACT

As top consumers in food chains, birds of prey forage over large geographical areas and so might be expected to accumulate environmental contaminants which are distributed in the environment. In this study, we investigated the concentration of Toxic elements (Cd, Hg, Ni, Pb and Cr) and essential elements (Zn, Cu and Co) in the outermost tail feathers of Black kite by ICPMS after wet digestion of the sample. Six out of eight metals were positively correlated with body weight, while two (Cr and Zn) are negatively correlated with weight. With length of feather Cd, Hg, Ni, Cu, Cr and Co were positively correlated and Pb and Zn were negatively correlated. Although feathers have been used intensively as a biomonitoring tool, few studies have addressed the effect of gender on the metal accumulation. We did not observe any significant gender difference in the metal concentration. Feathers are suitable indicators for monitoring heavy metal pollution and give us information about incorporation paths and ecotoxic effects. In this preliminary study, an attempt is made to compute and assess the impact of various trace elements from feathers of Black kite (*Milvus migrans govinda*) in arid zone of Gujarat.

KEY WORDS: **Black kite, Heavy metals, Feathers, ICPMS, Bio-indicator.**

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INTRODUCTION

Environmental contamination caused due to anthropogenic activities is a relatively novel concept, in ecological times. Stressors negatively affect individuals and entire ecosystems via air and water around the world, even in areas relatively free[1].However, in the past few decades, much efforts have been made regarding the monitoring of health of the ecosystem. Species that are at the apex of the food web are often used as bio-indicators to evaluate the presence of persistent contaminants in different ecosystems. As a result of escalating generation of evidences that bird populations are particularly sensitive to the changes produced by human in the environment, potential use of birds as indicator for environmental pollution has been widely recognized. Most of these species are long-lived, so pollutant burden gets integrated in a complex way over time. Birds are useful biomonitors for a number of reasons: they are visible, sensitive to environmental changes and they are at the pinnacle of the food chain, which makes them suitable for studying bioaccumulation. Their ecology, physiology and behaviour have been well studied and they are of interest to the public. Most studies have been conducted on internal tissues, but the number of studies making use of non-destructive methods, like measuring the concentrations in feathers, faeces and eggs, has increased over the years. Especially feathers may be valuable tool to monitor exposure to heavy metals. Raptors are amongst the most intensively studied bird species in biomonitoring investigations due to their apex position in the food chain and the spatial integration of contaminant levels in their extended home ranges. [2, 3, 4, 5] However, many raptors are extremely mobile, feeding over a wide geographical area making it complicated to determine the source and site for their toxic ingestion. The presence of pollutants, such as heavy metals, in the environment presents great risks for all living organisms, including humans. Heavy metals are frequent waste products of industrial activities and their emission often results in the contamination of the surrounding environment [6]. To detect the occurrence and the effects of heavy metals, monitoring programs, which measure the concentration in the different compartments of the environment and in biota, have been developed [7, 8]. Environmental toxicant like heavy metals can bind to the protein-molecules in the feather during the short period of feather growth when the feather is connected with the bloodstream through small blood vessels [7]. Birds excrete elements into growing feathers and elemental concentration in feathers can be higher and hence, easier to detect and quantify than the metals present in blood or other tissue sample [9, 10].

The Black Kite (*Milvus migrans govinda*) is a common diurnal scavenger distributed throughout the Paleartic region [11] while *M. m. govinda* is a very common scavenger around towns in India [12]. This species was much more abundant in the recent past and now has been classified as least concerned. The Black kite is migratory, medium sized raptor with a life history that makes it a species suitable for contaminant monitoring and heavy metal contamination has been suspected as an important factor contributing to the decline of the population of other races of this species. In this preliminary study an attempt is made to quantify and assess the impact of various trace elements from feathers of Black kite (*Milvus migrans govinda*).

MATERIALS AND METHODS

Sample collection

With the help of various NGOs and Forest department from Ahmadabad, Central Gujarat Black kite Primary feathers were collected from dead Adult kites (both Males and Females) during Kite Flying Festival of Gujarat when large numbers of birds are injured due to sharp threads. For the analysis of heavy metals we used 40 Black kites (20 males and 20 females). Three primary feathers were taken from each bird for the heavy metal analysis. Permission for sample collection was granted by the Forest Department, Government of Gujarat, Gandhinagar, Gujarat.

Sample treatment and analysis

The Primary feathers of Black kite were washed in order to remove external contamination, according to the procedure developed for human hair and nails samples [13, 14]. Feathers were washed alternately with Deionized water (Mili-Q) and acetone. These samples were exposed to 60 ^{0}C in dry oven for 24 h and the dry weight was determined. Feathers were digested in a 1:1 Mixture of Nitric acid (Qualigen) (70%) and Hydrogen Peroxide (Merck) (30%). The digestion was completed with the microwave destruction procedure described by Blast *et al* (1988) [15]. All samples were diluted by adding 4 ml deionized water (Milli Q) and were stored at -20 ^{o}C until analysis. Analysis was carried out by Inductively Coupled Plasma Atomic Mass Spectrometer (ICPMS).

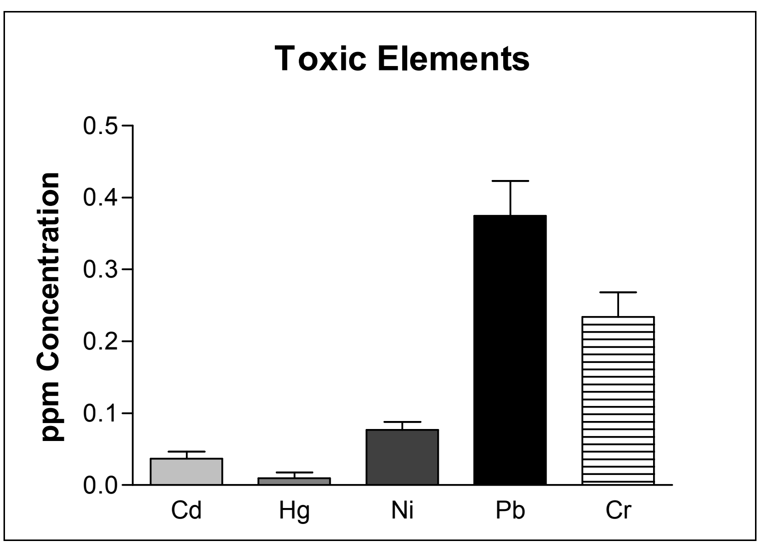
Statistical analysis

Statistical evaluation of the data was done using Statistical software package PAST (Mann Whitney *U* test) and SPSS 7.5 (Spearman correlation coefficient). Comparison between heavy metal in males and females was done by Mann Whitney *U* test. The relationship between body weight and length of feather with metal concentration were verified with Spearman correlation coefficient. Statistically significant level of probability was $p < 0.05$.

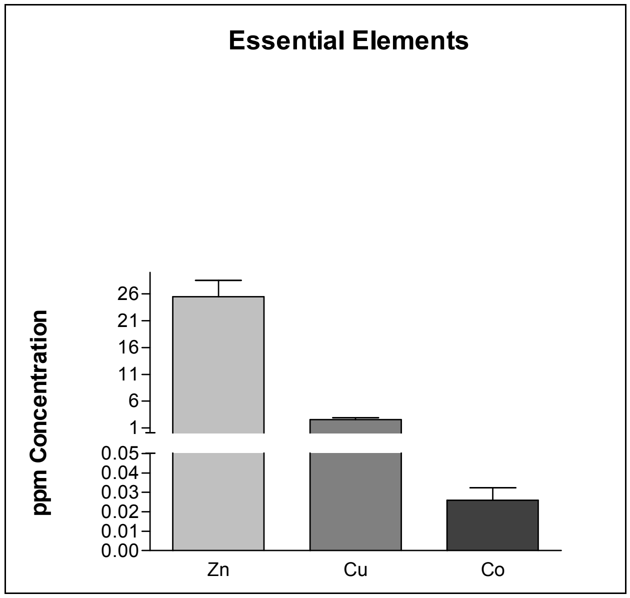
RESULTS

The mean concentration of Toxic elements (Cd, Hg, Ni, Pb, Cr) and Essential elements (Zn, Cu, Co) is shown in Graph: 1 and Graph: 2, while concentration of Essential and Toxic elements with respect to Body weight and length of feather are shown in Graph: 3 to 6.

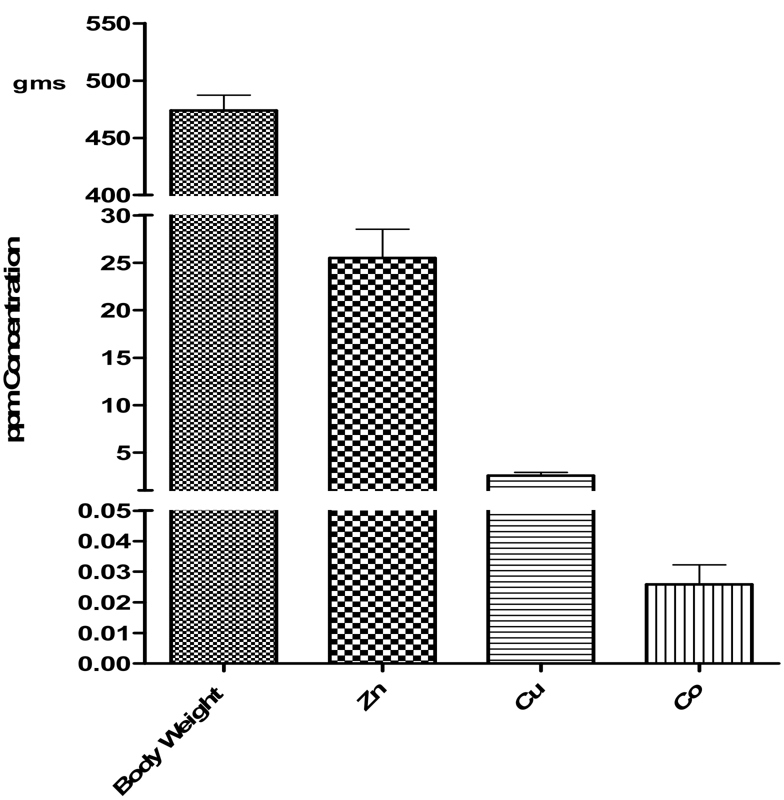
Graph 1
Concentration of Toxic elements (ppm) in Primary feathers



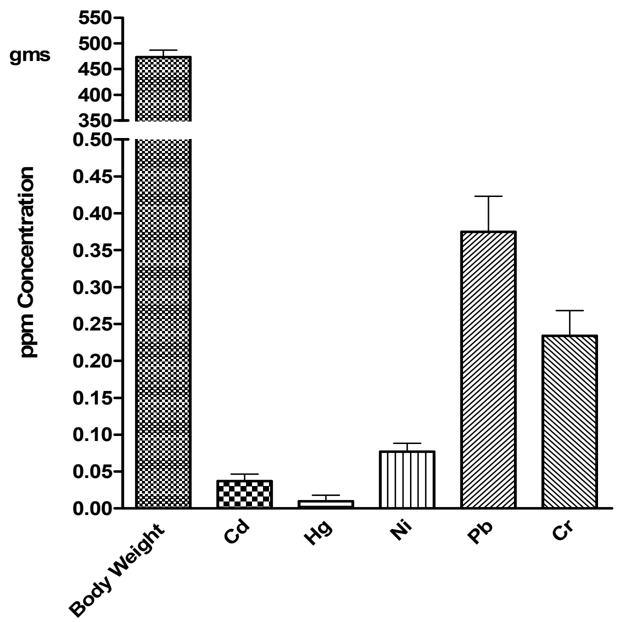
Graph 2
Concentration of Essential elements (ppm) in Primary feathers



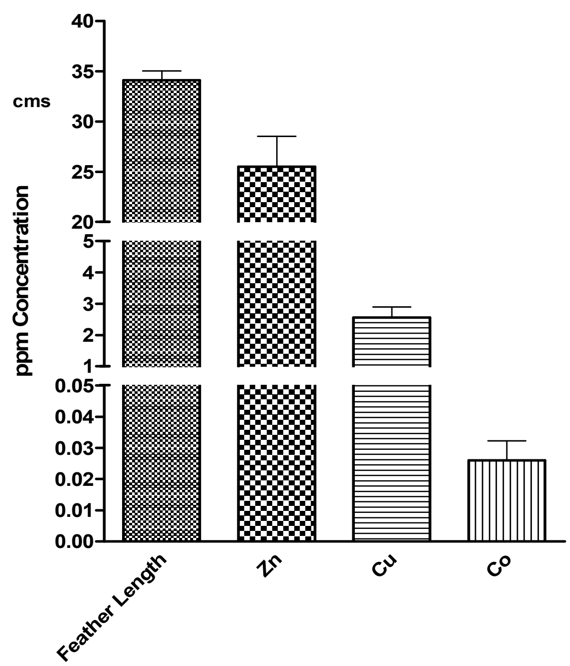
Graph 3
Accumulation of essential elements in primary feathers with respect to Body weight.



Graph 4
Accumulation of toxic elements in primary feathers with respect to Body weight.



Graph 5
Accumulation of essential elements with respect to Length of feather.



Graph 6
Accumulation of toxic elements with respect to Length of feather

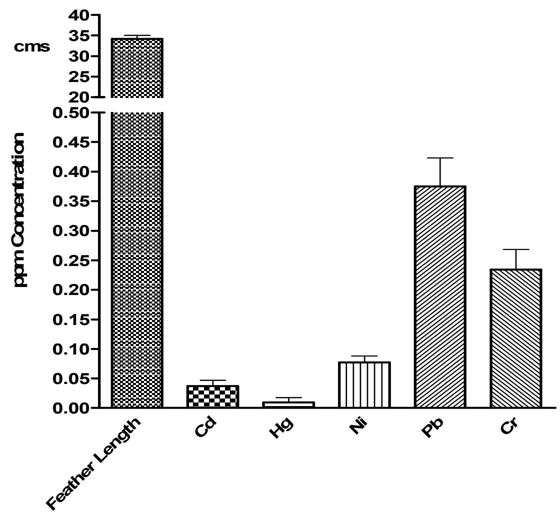


Table 1
Comparison of toxic and essential elements in males and females was made by Mann Whitney U test.

	Toxic metals					Essential metals		
Metals	**Cd**	**Hg**	**Ni**	**Pb**	**Cr**	**Zn**	**Cu**	**Co**
U	4	12.5	16	12	9	16	7.5	10
Z	-2.177	-0.80	-0.24	-0.88	-1.36	-0.24	-1.604	-1.205
P Value	0.02	0.42	0.80	0.37	0.17	0.81	0.108	0.22

Table 2
Spearman Correlation between Body weight and Length of feather with essential and toxic metals.

	Body Weight	Length	Cd	Hg	Ni	Pb	Cr	Zn	Cu	Co
Body Weight		0.810 **	0.162	0.631*	0.576*	0.061	-0.216	-0.294	0.043	0.573*
Length	0.810 **		0.85	0.217	0.222	-0.224	0.150	-0.161	0.075	0.617*

** P< 0.01, * P<0.05

Comparison of Heavy metals in males and females:
Our investigation indicates that there was no difference between levels of heavy metals in males and females as showing in Table: 1.

Correlation between body weight, length of feather and heavy metal accumulation:
Adverse effect threshold levels have not been established for these elements except Co in feathers. Insufficient data currently exists to predict hazardous levels of toxic metals in feathers. Feathers collected from urbanized study site contained elevated levels of Hg, Ni, and Co, though comparative data and information on the toxicological significance for

these elements in feathers are extremely limited. Spearman correlation coefficient was employed to examine the correlation between Body weight and length of feather with toxic and essential metal accumulation. Body weight was positively correlated and was significant with cobalt, nickel and mercury ($p<0.05$ Table: 2). While Length of feather was significant only with cobalt ($p<0.05$). Rest of the heavy metals showed significant correlation with the length of the feather.

DISCUSSION

Heavy metals are considered to be an important threat to the environment as they can accumulate within an ecosystem and endanger its health. However, unlike other sources that may cause population decline through individual mortality that are easy to detect, elevated levels of trace elements can affect many components without resulting in immediate death. For that reason heavy metals can be considered, except in extreme situations of pollution, as a source of silent death that is extremely difficult to detect. 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 disease [16]. Many studies have investigated the metal levels in feathers of birds and birds are used intensively for the past 30 years as a biomonitoring tool [7]. In the present study it is revealed that urbanization has very little impact on accumulation of heavy metal particularly in feathers and hence the median concentrations of these metals were markedly lower in feathers of Black kite (*M. m. govinda*) in Central Gujarat.

Relationship between Feather length, Body weight and concentration of heavy metals:
Accumulation of heavy metals in the feathers takes place in the period of their formation and the highest concentration is found in the distal part of a feather [17]. As Orlowski *et al* (2006) [18] describes that in the large feathers the load of contamination gets into feathers with the blood in the initial period of their formation, and then undergoes gradual reduction as a result of the growth of the feather. Length of feathers was statistically significant ($p<0.01$) with Body weight and Cobalt showed positive significant correlation with length of feather. The negative correlations found in between feather length and concentrations of Zinc and Lead. This shows that the concentration of heavy metal decrease as feather length increases. Among the toxic element Mercury and Nickel were significantly correlated with Body weight. As body weight increases, consumption of food also increases and the accumulation of metal is significant. Metal levels in feathers may, however, change and increase with age due to exogenous contamination. Exogenous contamination on to the feather surface may be caused by atmospheric depositions and/or may originate from secretion products of the uropygial gland smeared onto the feathers during preening. The crucial, statistically significant negative correlations between feather length and concentrations of Pb and Zn (for other metals non-negative and non-significant) show that the concentrations of heavy metals decrease as feather length increases. Accumulation of heavy metals in the feathers takes place in the period of their formation, and the highest concentration is found in the distal part of the feather. Most probably, in the large feathers, the load of contamination gets into feathers with the blood in the initial period of their formation, and then undergoes gradual reduction, as a result of the growth of the feather in the final period of growth.

Mercury concentration showed significant difference among various species of birds of prey and highest mercury accumulation was in sea birds [19]. Mercury concentration was high in aquatic birds positively due to dual exposure of the heavy metal through food and water while concentration of the same was low in terrestrial birds of prey. Hg is associated with several adverse effects, such as reduced food intake leading to weight loss; progressive muscle weakness in wings and legs with in-coordination and difficulty in flying, walking and standing; paralysis; convulsions; and death [20]. Mercury can also affect populations of free ranging birds through an increase in mortality and/or a decrease in fecundity and hatching and fledging rates [7].

Gender Difference:

The accumulation of heavy metals show no gender bias as demonstrated in the red- billed gulls (*Larus novaehollandiae scopulinus*) [21] and in Great tit (*Parus major*) and Blue tit (*Parus caeruleus*) [22]. In present study, also there was no significant gender difference for all metals while few studies have looked for differences in Mercury level between sexes. Burger and Gochfeld (1987) [23] found significant differences in levels of several metals between male and female ducks, although mercury levels were the same in both sexes. Hutton (1981) [24] found differences in levels of zinc and cadmium between male and female oystercatchers *Haematopus ostralegus.* In Mallards, Heinz (1976) [25] found higher mercury levels in the livers of males than in those of females after the egg-laying period, and Braune and Gaskin (1987) [26] found significantly lower levels of mercury in primary feathers of female Bonaparte's gulls when compared with primaries of male birds. Few studies have examined the effect of gender on the accumulation of heavy metals in feathers and other tissue [27, 28]. Nevertheless, there is reason to expect that metal levels may vary in those species that have sexual size dimorphism or have differential diets. Monitoring programs should be executed in order to identify elevated levels of toxic elements and to minimize their effect on the ecosystem. Biological monitoring is direct qualitative and quantitative assessment of exposure of a group of persons or individual to noxious agents present in the environment [29]. Advantages of using biomonitors are recently acknowledged and 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 level of trace elements in bird's organs and feathers may reveal the levels of toxic elements in their entire home range.

CONCLUSION

Feathers of raptors are potentially appropriate biomonitors for environmental heavy metal contamination. In this present study, significant correlation is seen between feather length and cobalt, body weight with mercury, cobalt and nickel respectively and other heavy metals assessed like cadmium, zinc, copper, lead and chromium showed no positive correlation with feather length and body weight. Gender bias accumulation of these metals is also not observed in Black kite. In conclusion, Black kites generally exhibited low to moderate concentrations of these elements in feathers. Based on these results, metal pollution does not appear to be an immediate threat to these birds. Black kites may be particularly well-adapted to survive in industrial areas. Based on the present findings, future study may wish to focus on assessing exposure to these elements on reproductive system and its potential effects on breeding status of these raptors.

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