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Heavy Metal Concentrations in Feathers of Critically Endangered Long-Billed Vultures (*Gyps Indicus*) in Bundelkhand Region, India

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ABSTRACT- Apart from the high trophic status, many birds of prey are territorial, non-migratory and live long lives, and so pollutant burdens recorded in soft body tissue, bones, feathers and eggs are likely to reflect chemical contamination within their extended home ranges. These local, upper trophic level species are believed to be especially vulnerable to metals and play a very important role as environmental contamination indicators. The concentration of Lead (Pb), Cadmium (Cd), Copper (Cu), Zinc (Zn) and Iron (Fe) were determined in feather samples of Long-billed vultures (*Gyps indicus*) (n = 100) collected from Tikamgarh, Lalitpur, Shivpuri, Chanderi and Panna Districts of Bundelkhand Region during 2007-2011. Sample preparation and analyses of metals were performed in the Indian Veterinary Research Institute, Izatnagar, Bareilly. Metal level in the samples was analyzed by Atomic Absorption Spectrophotometer (AAS 4141, ECIL, Hyderabad, India) wavelength of 229.5 nm (detection limit 0.005 µg/mL) and 217 nm (detection limit 0.025 µg/mL), respectively with 6mA current. The concentration of Cd, Pb, Cu, Zn and Fe determined in all the feathers collected from five sites was within the range of 0.1 µg/g–0.4 µg/g, 0.47µg/g–6.4 µg/g, 2.9 µg/g–8.11 µg/g, 9 µg/g–21.4 µg/g and 18.3 µg/g–194.9µg/g respectively According to ANOVA test, significant differences (0.05) were found for metals (Pb, Cd, Cu, Zn and Fe) in all feathers from Tikamgarh, Lalitpur, Shivpuri, Chanderi and Panna. When the metal toxicity results in feathers were seen district wise (Tikamgarh, Lalitpur, Shivpuri, Chanderi and Panna) no significant variance were reported in the occurrence of metals. No significant relationships were found between the metals in feather sample concentrations and their death. It can therefore be concluded that the metal concentrations are much below the lethal toxic levels and had no affect on the health of the dead vultures. The results of this study allowed concluding that the Long-billed vultures in the study area were not exposed to metal poisoning. The absence of linkage between the metal toxicity and vulture mortalities is not unexpected when consulted with other previous studies.

Key-words- Long-billed vultures, Feathers, Lead, Cadmium, Copper, Zinc, Iron

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INTRODUCTION

Birds can serve as bio-indicators of wider conditions [1] and they can even be used for predicting future environmental changes. In this sense, the use of wild birds as monitors of environmental contamination offers important information about the effects of the pollutants on these animals and also on the human species [2].

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Animals situated at the top of the food pyramid (for example, raptor species) can yield information over a large area around each sampling site, not only on bioavailability of contaminants but also on how, where and when they are transferred within the food chain [3]. The impact of heavy metals on the environment can be a serious threat to the stability of the ecosystem [4].The nonessential heavy metals lead (Pb) and cadmium (Cd) are emitted and globally distributed mainly through industry, road traffic, and consumption of fossil fuels [5] and their effects on animal health has been clearly established. Lead poisoning and high lead exposure in birds has been a concern for more than a century [6], and cadmium has been described as one of the most dangerous trace elements in food and in the environment [4]. Other heavy metals, such as zinc (Zn) are essential, required to support biological activities, but when their environmental concentrations rise, they can generate serious toxicological problems. All these heavy metals can hamper the reproductive output or even cause death [7], constituting a serious threat to the survival of wild bird species [8]. As a result of bioaccumulation, raptor species can accumulate high levels of metals, and it is known that once metals enter a bird, these elements can be stored in internal tissues such as the kidney and the liver [9].

Apart from their high trophic status, many birds of prey are territorial, non-migratory and live long lives, and so pollutant burdens recorded in soft body tissue, bones, feathers and eggs are likely to reflect chemical contamination within their extended home ranges. These local, upper trophic level species are believed to be especially vulnerable to metals [10] and play a very important role as environmental contamination indicators [6,11] because of this vulnerability to a great variety of environmental contaminants, birds of prey have been used extensively as biomonitors of environmental quality [12]. Heavy metals, both essential and non-essential, have the potential to be toxic to organisms above a certain threshold concentration. The threshold concentration depends on various factors, including the bird species, the metal type, bioavailability and exposure [13].

In the present study, blood samples were not used for the presence of heavy metals. The *Gyps* species are listed as critically endangered and so no attempt was made to collect blood samples. Consequently, collection of ecotoxicological data on protected raptors has been made very difficult and tedious. This is the reason why knowledge on predacious bird eco-toxicology progresses relatively slowly. During the recent years the use of feathers in biomonitoring programmes has increased. Burger in 1993 gave several reasons why feathers are useful for determining metal levels in birds:

- Metals are deposited in the feathers during their formation.
- Metals are only deposited during the short period of feather growth and are thus a record of blood levels at the time of feather formation.
- Feathers are easy to collect, as they can be plucked from both live and dead specimens.
- Feathers are collected from live birds without causing undue damage.
- Collection from live birds is a non-invasive procedure that can be performed by field assistants.
- Feathers can be easily stored in metal-free containers and do not require refrigeration.
- Metal profiles are not easily disrupted by long-term storage.

The present study was aimed to determine the contents of 3 essential trace metals (zinc, copper, and iron) and those of two highly toxic ones (lead and cadmium) in tissues of the *Gyps* vultures from Bundelkhand Region.

MATERIALS AND METHODS

The most easily collected samples were the molted feathers from various vulture colonies (2007-2011). Sample preparation and analyses of metals were performed in the Indian Veterinary Research Institute, Izatnagar, Bareilly.

Preparation of feathers

- Feather biometrics was done i.e. the length (whole feather), length of stalk and width of all the collected feathers was taken.
- Feathers were first washed with distilled water and then with acetone.
- The feathers were kept in hot air oven at 70^0C for about 4 hours.
- The feather was cut and equal portion of upper, middle and lower portion were mixed and 0.5gm/5mg of each sample was kept in labeled containers/bags.

Digestion of samples

- The samples were transferred to labeled conical flask of 100 ml.
- Wet digestion was done with acid mixture (nitric and perchloric acids in the ratio of 1:4). 10 ml acid mixture was added to each sample.
- Two analytical blanks were run simultaneously with each batch of digestion with de-ionized triple distilled water as biosample.
- Equal amount of acid mixture was added in blanks during digestion.
- The samples were kept over night in the acid mixture for digestion.
- The samples were kept in a separate well ventilated room so that the vapours pass out.

Sample preparation for Atomic Absorption Spectrophotometer (AAS) analysis

- After digestion was completed, the flasks were kept on hot plate at 200^0C till 0.5 ml of solution was left.
- The evaporation was done in a well ventilated room.
- The sample was then filtered.
- In each filtrate distilled water was added to make it 5 ml.
- Metal level in the samples was analyzed by Atomic Absorption Spectrophotometer (AAS 4141, ECIL, Hyderabad, India) wavelength of 229.5 nm (detection limit 0.005 µg/mL) and 217 nm (detection limit 0.025 µg/mL), respectively with 6mA current.
- The measurements of detection limit were obtained by taking twice the standard deviation of measurement of blank samples.

RESULTS AND DISCUSSION

The results are in accordance with study of [14-16]. Correlation between heavy metal levels was determined. Intergroup differences were tested using one-way analyses of variance (ANOVA) and the Pearson correlation to test the relationship between variables. Results were analyzed and compared with human and published animal reference values. The feathers are non-destructive biomonitors that have been used broadly for heavy metal detection. The heavy metals in blood bind to the keratin in feathers. When the birds molt the growing feathers, the heavy metals are excreted with them. Thus the molted feathers contain information about the circulation of heavy metals in the blood during the development stages. This is endogenous contamination. When exposed to external environment, the concentration of heavy metals may change, known as exogenous contaminations. This in turn elevates the heavy metal concentrations. The feathers are easy to collect and store at room temperature.

Cadmium (Cd) is known for its long half-life in biological systems (decades in humans and years in birds). Cadmium is a teratogen, carcinogen and possible mutagen [17]. Adverse effects in fish and wildlife are probable when Cd concentration exceeds 3 mg/kg (ppb) in freshwater, 4.5 mg/kg in saltwater, 1000 ppb in the diet, or 100 mg/m3 in air [17]. According to the ANOVA Test, significant differences (P=0.05) were found for all metals in feathers from Tikamgarh [F=147. 169; P=0] in Madhya Pradesh (Table 1), Lalitpur [F=122. 173; P=0] in Uttar Pradesh (Table 2), Shivpuri [F=103. 169; P=0], Chanderi [F=85. 480; P=0] and Panna [F=278. 597; P=0] again in Madhya Pradesh (Table 3-5). The concentration of Cd determined in all the feathers collected from Tikamgarh, Lalitpur, Shivpuri, Chanderi and Panna was within the range of 0.1µg/g-0.4 µg/g (Table 6, Graph 1).

Lead (Pb) intoxication is one of the most commonly reported and clinically recognized poisonings of companion and free-ranging birds. Lead in bone has been physiologically incorporated over the lifetime of the bird, whereas feather lead comprises both that physiologically incorporated at the time of feather formation, and lead deposited on the feather surface between formation and moult or sampling (usually one to several years in the wild –longer with museum specimens). This makes physiologically incorporated or endogenous lead difficult to identify in feathers [18], thus may limit their utility for monitoring lead absorption. However, feather sampling is simple and non-invasive, and could potentially be of use for future studies. Like Cadmium, lead is an element that plays no role in metabolic processes of animal organisms. It is an extremely toxic element with a wide range of harmful effects.

Table 1: One way ANOVA for feathers from Tikamgarh (T1 to T25)

Test of Homogeneity of Variances			
Levene Statistic	**df1**	**df2**	**Sig.**
53.390	4	120	0.000

ANOVA					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	266338.321	4	66584.580	147.169	0.000
Within Groups	54292.319	120	452.436		
Total	320630.640	124			

Table 2: One way ANOVA for feathers from Lalitpur (L1 to L25)

Test of Homogeneity of Variances			
Levene Statistic			
31.497	4	120	0.000

ANOVA					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	243131.626	4	60782.906	122.173	0.000
Within Groups	59701.816	120	497.515		
Total	302833.442	124			

Table 3: One way ANOVA for feathers from Shivpuri (S1 to S19)

Test of Homogeneity of Variances			
Levene Statistic	**df1**	**df2**	**Sig.**
37.080	4	90	0.000

ANOVA					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	157566.872	4	39391.718	103.980	0.000
Within Groups	34095.554	90	378.839		
Total	191662.426	94			

Table 4: One way ANOVA for feathers from Chanderi (C1 to C10)

Test of Homogeneity of Variances			
Levene Statistic	**df1**	**df2**	**Sig.**
35.309	4	45	0.000

ANOVA					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	80442.414	4	20110.604	85.480	0.000
Within Groups	10587.031	45	235.267		
Total	91029.445	49			

Table 5: One way ANOVA for feathers from Panna (P1 to P10)

Test of Homogeneity of Variances			
Levene Statistic	**df1**	**df2**	**Sig.**
18.581	4	45	.000

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	151418.807	4	37854.702	278.597	.000
Within Groups	6114.440	45	135.876		
Total	157533.247	49			

Table 6: Correlation between various metals in feathers

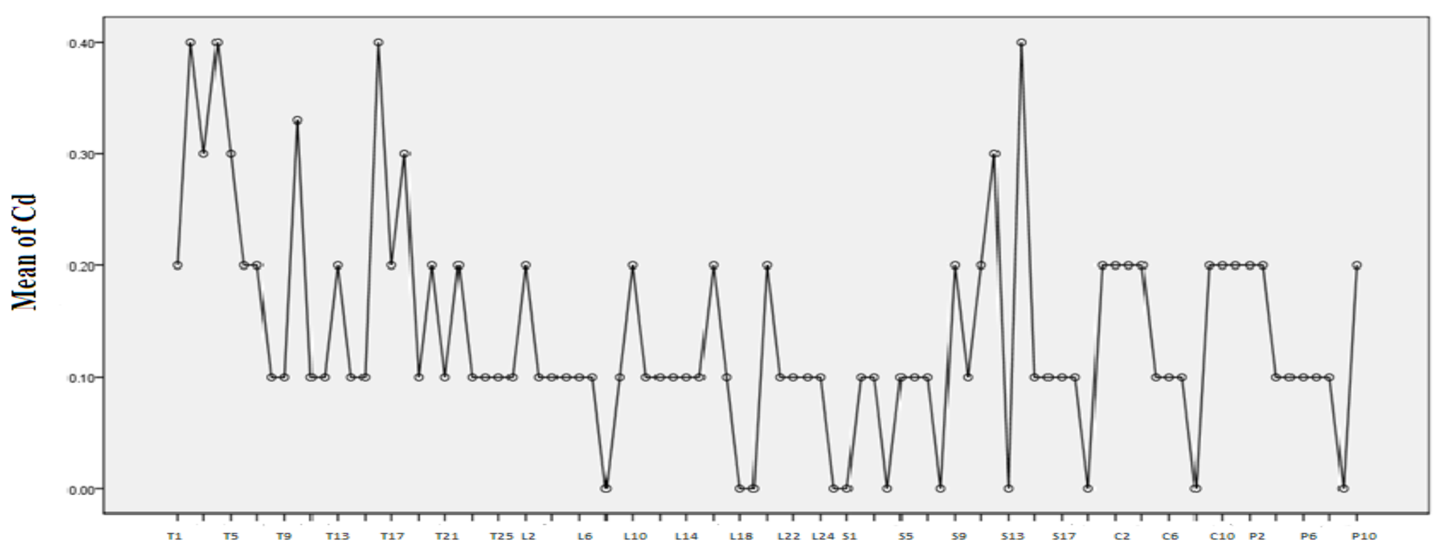
Correlations

		Pb	Cd	Cu	Zn	Fe
Pb	**Pearson Correlation**	1	.384**	.000	-.007	.073
	Sig. (2-tailed)		.000	.998	.946	.498
	N	89	89	89	89	89
Cd	**Pearson Correlation**	.384**	1	.049	.032	.267*
	Sig. (2-tailed)	.000		.649	.765	.011
	N	89	89	89	89	89
Cu	**Pearson Correlation**	.000	.049	1	.126	.203
	Sig. (2-tailed)	.998	.649		.240	.056
	N	89	89	89	89	89
Zn	**Pearson Correlation**	-.007	.032	.126	1	.348**
	Sig. (2-tailed)	.946	.765	.240		.001
	N	89	89	89	89	89
Fe	**Pearson Correlation**	.073	.267*	.203	.348**	1
	Sig. (2-tailed)	.498	.011	.056	.001	
	N	89	89	89	89	89

**Correlation is significant at the 0.01 level (2 tailed)

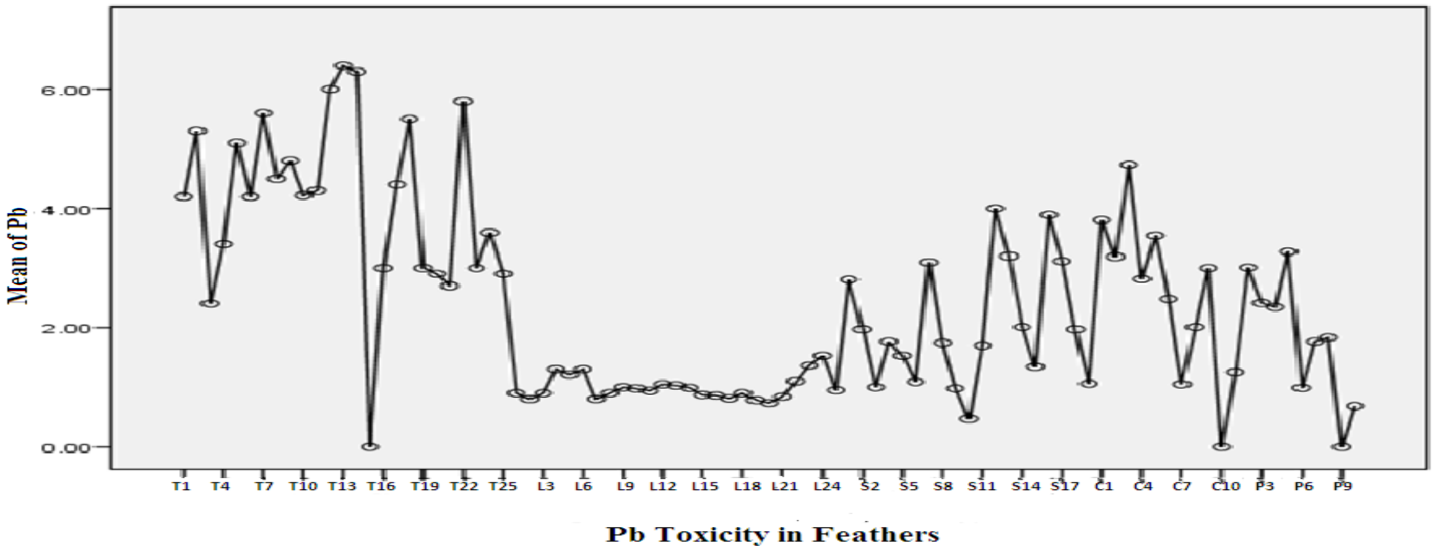
*Correlation is significant at the 0.05 level (2-tailed)

[Pb-Lead; Cd-Cadmium; Cu-Copper; Co-Cobalt; Zn-Zinc; Fe-Iron]



Graph 1: Mean of Cadmium toxicity in feathers

The concentration of Pb determined in all the feathers collected from Tikamgarh, Lalitpur, Shivpuri, Chanderi and Panna was within the range of 0.47µg/g-6.4 µg/g (Graph 2). However, there were no clinical signs of lead toxicosis and there were no pathological or physiological changes. The vultures in the study area are not exposed to lead when feeding on the remains of animals as there is no shooting with lead ammunition. Although the lead concentration determined during the study was low than the highest permissible level, it must be remembered that even as little as 0.16 mg Pb/Kg body weight of the bird has a toxic effect on its organism. When compared with the declared values (Table 7), Pb was found to be below toxic levels. No pathological changes from Pb exposure were found in the organs such as kidney, heart, liver, and muscle. The growth of vultures was not retarded and no pathological changes and physiological dysfunction were found.

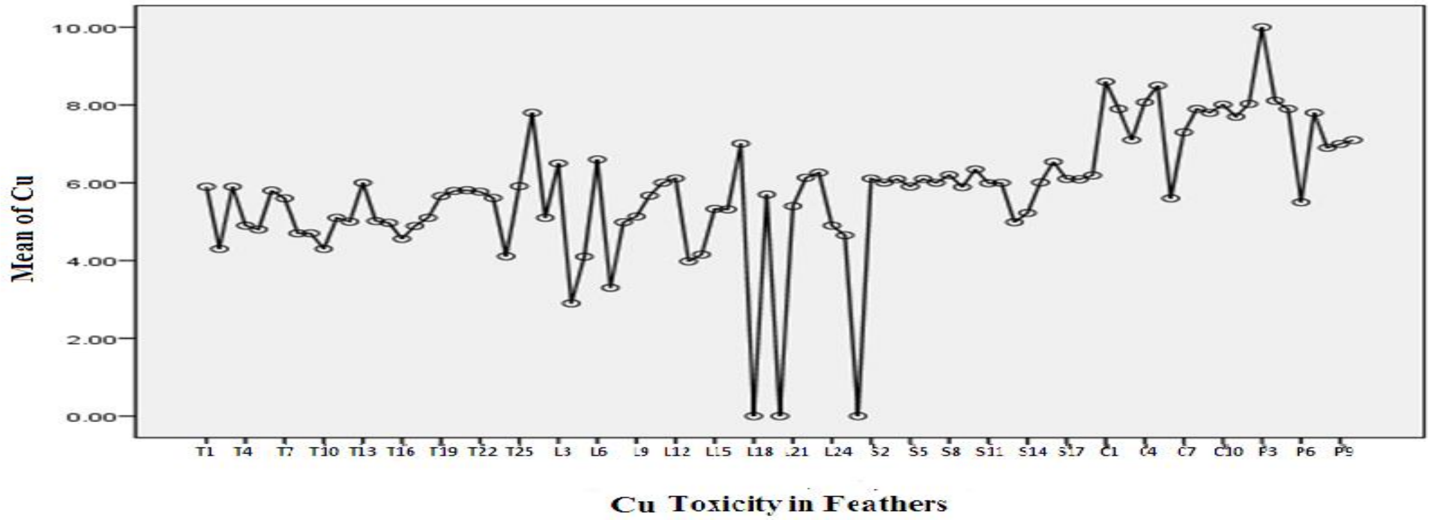


Graph 2: Mean of Lead toxicity in feathers

Table 7: Declared values (CR) and their study's results (OR) of trace metal contents in the certified reference material BCR No. 186 (lyophilised pig kidney) (data expressed as µg/g dry weight) [16]

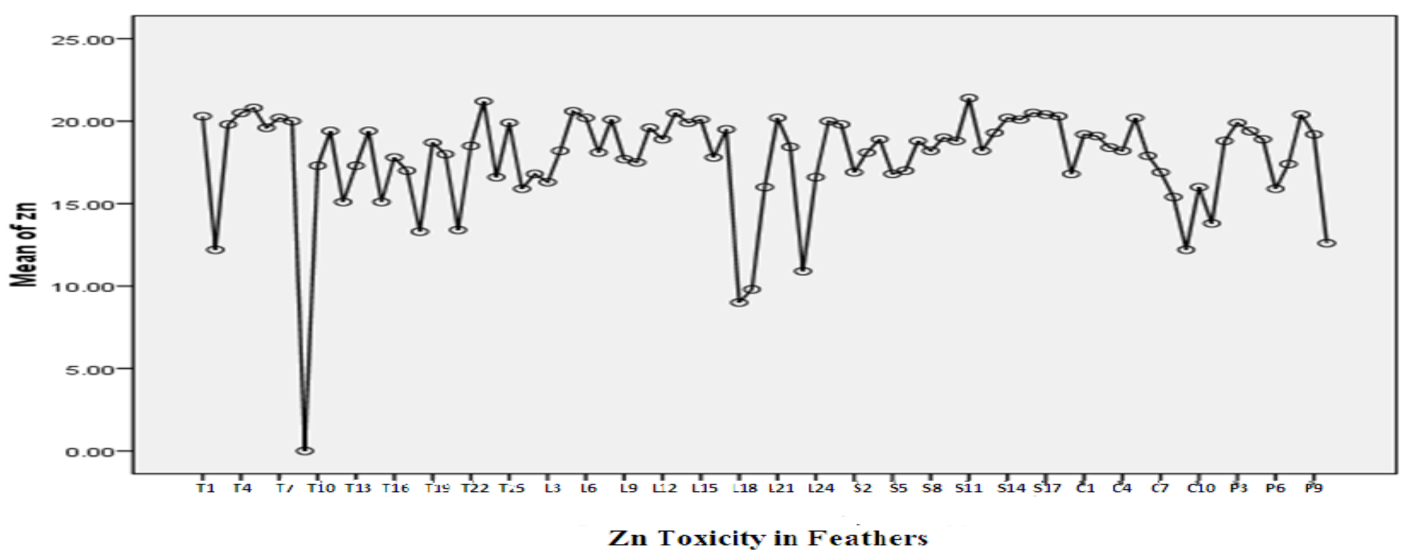
Value	Fe	Mn	Cu	Zn	Pb	Cd
CR	299 ± 10	8.5 ± 0.3	31.9 ± 0.4	128 ± 3	0.306 ± 0.011	2.71 ± 0.15
OR	327 ± 9	9.1 ± 0.1	34.0 ± 0.3	131 ± 2	0.412 ± 0.161	3.30 ± 0.06

Copper (Cu) is plentiful in the environment and essential for the normal growth and metabolism of all living organisms [19,20]. Abnormal levels of copper intake may range from levels as low as to induce a nutritional deficiency to levels as high as to be acutely toxic [21]. Copper is an essential component of the animal system and plays an important physiological role in haematopoiesis, myelin formation, phospholipids formation, connective tissue metabolism and enzyme systems. The concentration of Cu determined in all the feathers collected from Tikamgarh, Lalitpur, Shivpuri, Chanderi and Panna was within the range of 2.9µg/g- 8.11µg/g (Graph 3). Copper toxicosis is rare in birds. It seems that external contamination of Cu in vultures is unrelated when judged against the bioaccumulation rate. In literature, contrasting results about exogenous and endogenous origin of Cu in feathers can be found. Copper signal along the feather might vary as effect of Cu levels in the diet during feather growth [22], and concentrations in growing feathers of chicks follows the accumulation of copper in interna tissues with age [23]. However, there were no clinical signs of Cu toxicosis and there were no pathological or physiological changes. The vultures in the study area are not exposed to copper toxicity.



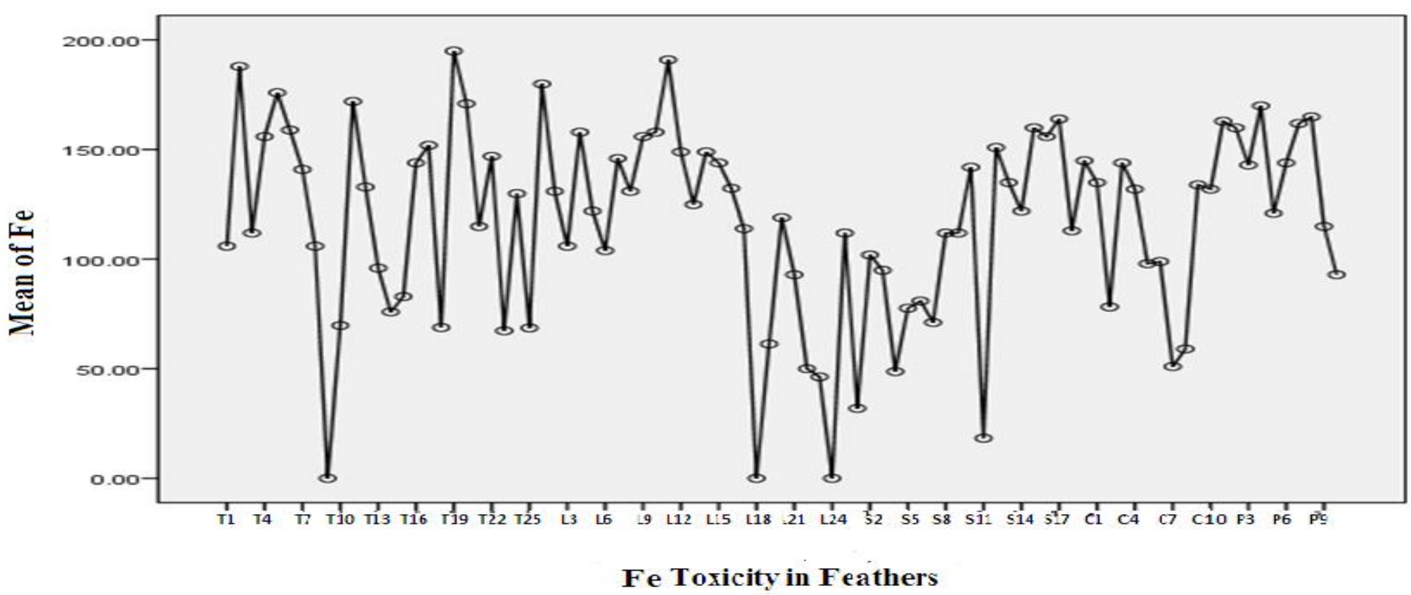
Graph 3: Mean of Copper toxicity in feathers
[T-Tikamgarh; L-Lalitpur; S-Shivpuri; C-Chanderi; P-Panna]

Zinc is an essential trace element for all living organisms. Zn has an important role in many metabolic processes, especially in the activation of enzymes and the regulation of gene expression [24]. Therefore its higher concentration may impair physiological functions of birds, as well as contributing to decline in species populations. The pancreas and bones are primary targets in birds and mammals. The balance between excess and insufficient zinc is important. Zinc is required for normal feather formation. Zinc deficiency has severe adverse effects on all stages of growth, development, reproduction, and survival. Avian diets should contain >25 mg Zn/kg DW ratios for prevention of zinc deficiency effects and <178 mg kg DW for prevention of marginal sub-lethal effects. The concentration of Zn determined in all the feathers collected from Tikamgarh, Lalitpur, Shivpuri, Chanderi and Panna was within the range of 9 µg/g - 21.4µg/g (Graph 4). However, there were no clinical signs of Zn toxicosis and there were no pathological or physiological changes. The vultures in the study area are not exposed to Zn as there are no major sources of anthropogenic zinc discharges to the environment that includes electroplaters, smelting and ore processors, drainage from active and inactive mining operations, domestic and industrial sewage, combustion of fossil fuels and solid wastes, road surface runoff, corrosion of zinc alloys and galvanized surfaces, and erosion of agricultural soils [25,26]. During smelting, large amounts of zinc are emitted into the atmosphere but there are no such activities in the study area. Most companion birds have acceptable liver zinc concentrations of 30 to 70 ppm (mg/kg) wet weight, and liver zinc concentrations of upto 100 ppm (mg/kg) expressed as wet weight are considered nontoxic. Once liver zinc concentrations exceed 100 ppm, zinc poisoning may be present and careful histological evaluation is necessary for a definitive diagnosis.

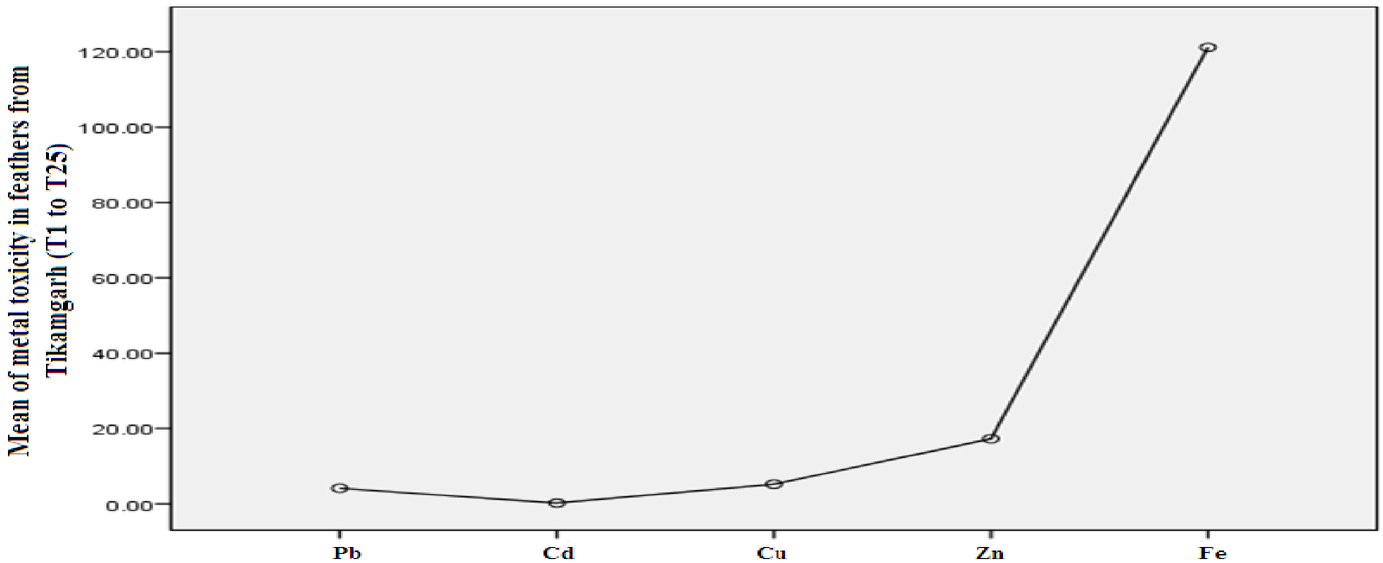


Graph 4: Mean of Zinc toxicity in feathers
[T-Tikamgarh; L-Lalitpur; S-Shivpuri; C-Chanderi; P-Panna]

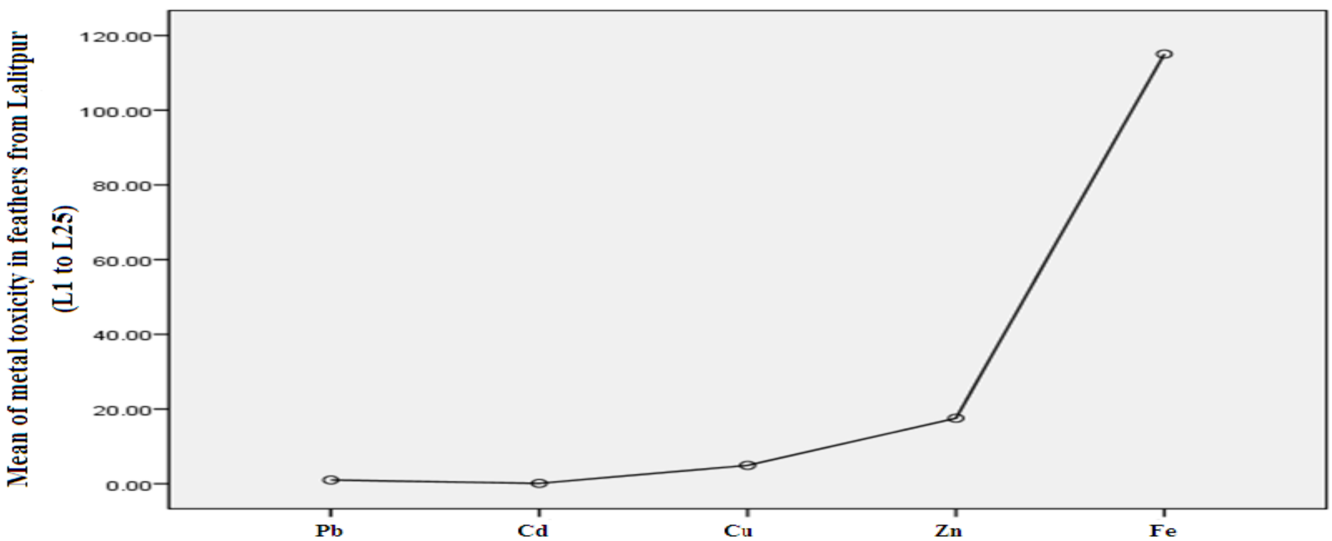
Iron (Fe) is a vital element in life. The major scientific and medical interest in iron is as an essential metal, but toxicological considerations are important in terms of accidental acute exposures and chronic iron overload. With rare exceptions, virtually all live organisms are dependent on iron for survival. Iron and its compounds present as pollutants in the atmosphere, can cause deleterious effects to humans, animals, and materials. Iron is a natural component of soils, but its concentration can be influenced by some industries. It has been reported that urban soils showed different heavy metal characteristics [27]. The concentration of Fe determined in all the feathers collected from Tikamgarh, Lalitpur, Shivpuri, Chanderi and Panna was within the range of 18.3µg/g–194.9µg/g (Graph75). All birds need some iron, to create blood and other tissues. Diets should be formulated to contain between 50 and 100 mg/kg iron, on a dry matter basis. Some authors, however, have recommended levels of 50-65 mg/kg, for diets fed to susceptible species [28]. The vultures in the study area are not exposed to Fe. The feathers tested for Fe toxicity showed a significant difference within the districts as well as between two districts. However the other four metals in feathers (Zn, Cu, Cd, Pb) showed little or no variations within and between the districts. According to ANOVA test, significant differences (0.05) were found for metals (Pb, Cd, Cu, Zn and Fe) in all feathers (T1-T25) from Tikamgarh district (F=147.169; P=0.000). Similarly significant differences (0.05) were found for metals (Pb, Cd, Cu, Zn and Fe) in all feathers (L1-L25) from Lalitpur district [F=122.173; P=0.000] as well as in all feathers (S1-S19) from Shivpuri [F=103.980; P=0.000]. Likewise, significant differences (0.05) were found for metals (Pb, Cd, Cu, Zn and Fe) in all feathers (C1-C10) from Chanderi district [F=85.480; P=0.000] as well as in all feathers (P1-P10) from Panna [F=278.597; P=0.000]. When the metal toxicity results in feathers were seen district wise (Tikamgarh, Lalitpur, Shivpuri, Chanderi and Panna) no significant variance were reported in the occurrence of metals (Graph 5-10). No significant relationships were found between the metals in vulture sample concentrations and their death. In the past feathers of mostly seabirds and bird of prey have been used as bio-indicators of metal pollution. The levels of various metals have been compared in the primary wing feathers of several birds of prey [29]. Bearded vultures in captivity are probably susceptible to lead poisoning. Dead wild animals delivered to the Zoos should, therefore, be x-rayed before being fed. Mortality from eating prey animals with lead shots embedded in their tissues or the gizzards of birds with ingested lead shot accounts for an estimated 10-15% of the recorded post-fledgling mortality in the two species [30].



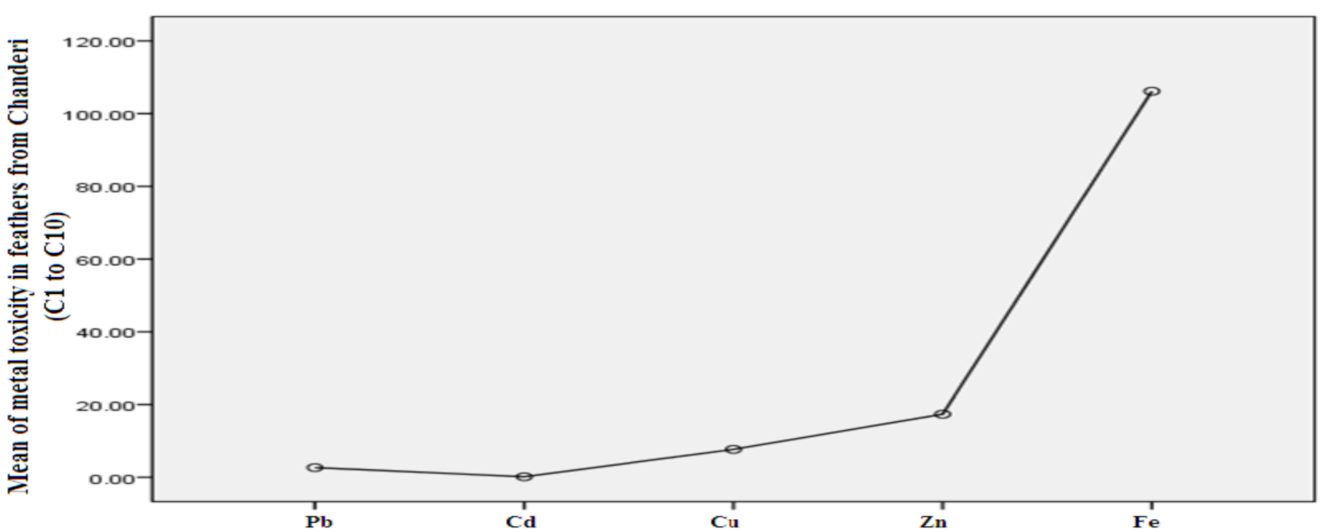
Graph 5: Mean of Iron toxicity in feathers.
[T-Tikamgarh; L-Lalitpur; S-Shivpuri; C-Chanderi; P-Panna]



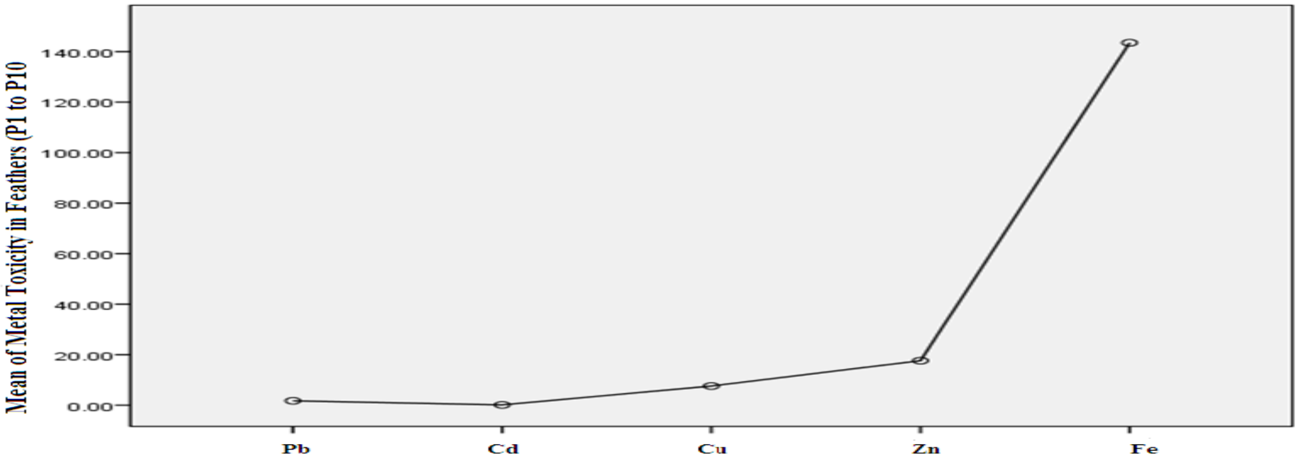
Graph 6: Mean of metal toxicity in feathers from Tikamgarh (T1 to T25)



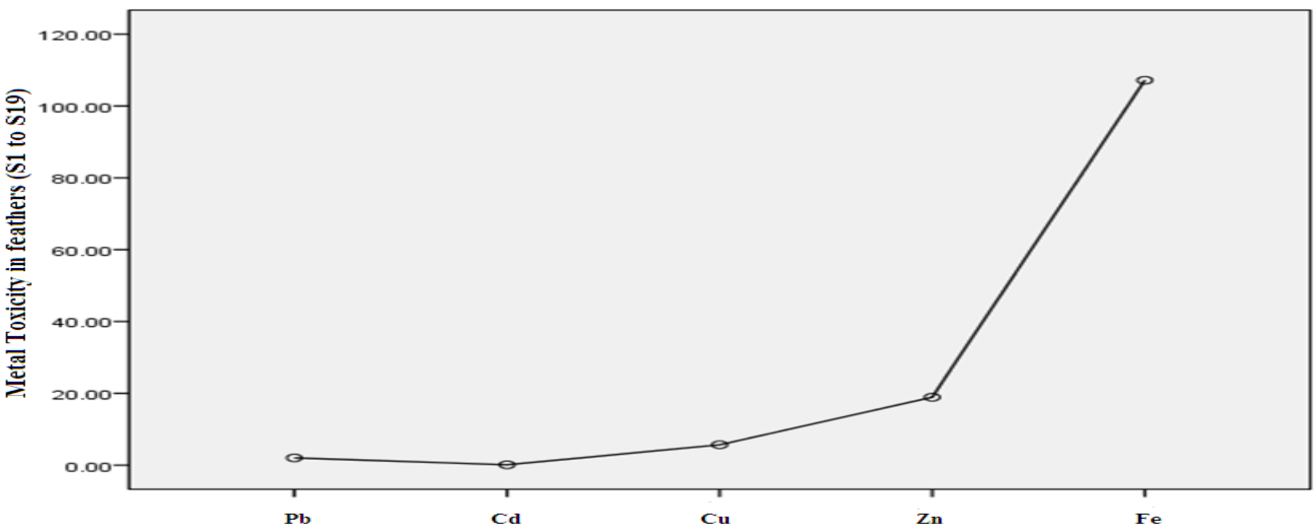
Graph 7: Mean of metal toxicity in feathers from Lalitpur (L1 to L25)
[Pb-Lead; Cd-Cadmium; Cu-Copper; Co-Cobalt; Zn-Zinc; Fe-Iron]



Graph 8: Mean of metal toxicity in feathers from Chanderi (C1 to C10)



Graph 9: Mean of metal toxicity in feathers from Panna (P1 to P10).
[Pb-Lead; Cd-Cadmium; Cu-Copper; Co-Cobalt; Zn-Zinc; Fe-Iron]



Graph 10: Mean of metal toxicity in feathers from Shivpuri (S1 to S19)

CONCLUSIONS

From the results of this study, feathers can be used as indicators of environmental contamination from metals and their adverse effect on living beings. It can therefore be concluded that the metal concentrations are much below the lethal toxic levels and had no affect on the health of the dead vultures. The absence of linkage between the metal toxicity and vulture mortalities is not unexpected when consulted with other previous studies. Due to scientific and conservation-related cause it is necessary to analyse occurrence of metals (essential and non-essential) and in-depth studies of the association between them.

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