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Assessment of Nickel and Chromium Concentrations in Black Kite (*Milvus migrans*) Tissues

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Abstract. A study was conducted to determine nickel (Ni) and total chromium (Cr) concentrations in liver and breast muscle of 24 black kites (*Milvus migrans*) collected from northern Punjab, Pakistan. The main objective of the study was to quantify residues of these metals and provide indirect information regarding the extent of contamination of their habitats. Overall average Ni and Cr were higher ($P < 0.001$) in breast muscle than liver. Correlation analysis indicated that Ni and Cr were significantly different ($P < 0.05$) in liver tissues in Kotla, whereas, all other combinations at both sites (Gujrat and Kotla) were non-significantly ($P > 0.05$) correlated.

Keywords: *Milvus migrans,* metal residues, avian predators, correlation analysis

Introduction

The black kite *(Milvus migrans)* is a widespread bird-of-prey that opportunistically exploits a wide array of food sources for instance, it captures live prey such as insects, reptiles, birds and small mammals throughout nearly all habitats from desert to forest and near rivers (Scheider *et al.,* 2004; Shiraishi *et al.,* 1990). In Pakistan, these kites exist throughout Punjab and their range varies by season (Altaf *et al.,* 2013). Raptors (e.g. black kite) are considered good bioindicators for several reasons, *viz.,* they are common, widespread, conspicuous, show toxic effects for environmental pollutants and are more sensitive to environmental changes (Furness, 1993). Predatory species of birds occupy the top of the food chain; hence they tend to bioaccumulate certain pollutants, and therefore, are used to monitor them. Furthermore, yield information over a large area around each sampling site is very important for bioavailability of contaminants as well as how, where and when they are transferred within the food chain (Zaccaroni *et al.,* 2008; Jager *et al.,* 1996).

In Pakistan, the rapid rise in human population, industrialisation and agriculture practices have major impacts on environmental contamination (Abbas *et al.,* 2012; Chandra and Kulshreshtha, 2004; Horrigan *et al.,* 2002; Baluch, 1995). Heavy metals are one of the pollutants and contaminate the environment through various routes *viz.,* mining, smelting, agricultural and natural activities (Navarro *et al.,* 2008; Vaalgamaa and Conley, 2008; Brumelis *et al.,* 1999). Chemical and metallurgical industries are the most important sources of heavy metals in the environment (Cortes *et al.,* 2003; Stawarz *et al.,* 2003; Raji and Anirudhan, 1997).

Although, some of the heavy metals are needed by organisms in trace amounts, but when present in excess, they can cause enzymes to denature, interact with nuclear proteins and DNA, causing oxidative deterioration of biological macromolecules and hampering the reproductive output and even causing death (Leonard *et al.,* 2004; Sanpera *et al.,* 2000; Chapman and Reiss, 1999). When either dietary concentrations or exposure times are long enough, some trace metals have the potential to reach toxic levels in the kidney (Nordberg, 1978; Friberg, 1952). The metals such as copper, manganese, nickel and zinc are regulated strongly by invertebrates because they are essential to many enzymes, while some metals are not essential including cadmium, chromium and lead. Metals accumulation may be a serious threat to the survival of wild birds and other organisms (ASTDR, 2005; Hernandez *et al.,* 1999).

The metal residues in plant and animals represent a balance between rates of uptake, detoxification, storage and excretion. These depend on the metal, diet, and physiology. Nickel is essential to birds, but chromium

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is not (Furness *et al.,* 1990). Chromium is a genotoxic can act as cancer agent when directly inhaled. Moreover, chromium compounds are very corrosive and can severely burn the skin. Such burns make it easier for chromium to be absorbed through the skin with the potential for causing systemic toxicity (Guertin, 2004). Whereas, nickel has various adverse health effects due to exposure and its compounds cause skin allergies, lung fibrosis, and lung cancer (Zhao *et al.,* 2009). Keeping in view the impact of metal accumulation in birds, the present study was designed to assess nickel and chromium level in the keel muscles and livers of male and female black kite from Gujrat district (northern Punjab), Pakistan.

Materials and Methods

The present study was conducted at two sites (a) Gujrat (34°17'26.05"N; 72° 9'39.23"E), an ancient district of Pakistan located between two famous rivers, i.e., Jhelum and Chenab, and (b) at Kotla Arab Ali Khan (32°51'7.63"N and 74° 4'23.57"E), a town and Union Council of Tehsil Kharian. Chromium and nickel concentrations have been increased in recent years due to human activities. For instance human activity contributes to Cr in the environment (air, surface water, groundwater, soil) from chromium plating, chemical manufacturing of chromium, and evaporative cooling towers (ATSDR, 2005). Combustion of coal and oil also release large quantities of chromium (ATSDR, 2005). Nickel and nickel compounds are widely used in making metal coins and jewellery and in industry for making items such as valves and heat exchangers. Most of the nickel is used to make stainless steel. There are also compounds consisting of nickel combined with many other elements, including chlorine, sulphur and oxygen (Zhao *et al.,* 2009).

A total of 24 black kites were collected (March to August 2013) from Gujrat and Kotla Arab Ali Khan (both were reference sites), 12 from each, comprising of 13 females and 11 males, out of which 14 were adults and 10 were sub adults. They were randomly collected by gun shooting, then weighed, measured, and dissected. Organs were preserved in 70% ethyl alcohol for later analysis.

Physical conditions were examined prior to necropsying to determine the health of specimens. Specifically, wet weight (wwt, g), and tarsus length (cm), were examined. Then, samples of keel muscle i.e., lungs, liver, heart, spleen, and trachea, all parts of gastrointestinal tract, kidneys, ovary and brain were taken in aluminum foil and shifted to ultra-low freezer at -80 °C and metal residues in liver and breast muscles were reported. Liver and breast muscle samples were digested in reagent grade nitric acid (70% concentration). Digested samples were subsequently diluted in de-ionised water and analysed for Cr and Ni using atomic absorption spectroscopy (Perkin Elmer 400) in the chemistry lab, university of Gujrat, Gujrat, Pakistan. The data obtained is presented in wet weight basis.

Statistical analysis. The data was first log transformed, then analysis of variance was applied to compare gender, location, metals and tissues. Moreover, correlations between nickel and chromium, breast muscle and liver were applied by using SPSS (Statistical software 13.0 version). The 0.05 level of significance was used for all statistical analysis.

Results and Discussion

Table 1 shows the comparison of heavy metals *viz.,* Ni and Cr concentrations in breast muscles and livers of black kite at two localities (Kotla Arab Ali khan and Gujrat). Overall male birds quantitatively contained lower concentrations of both Ni and Cr in their breast muscles compared to females at both sampling sites. At Kotla, male birds contained 36.12±5.84 mg/L and female 38.75±6.18 mg/L concentrations of Ni in their breast muscles and almost similar trends were recorded for Ni concentration both in males and females breast muscles in Gujrat. Very interestingly, irrespective of Ni concentration, Cr concentration was recorded higher in female breast muscles (2.04±0.07 mg/L) than males at Kotla. Nickel and Cr concentrations were higher in livers of males and similar trends were found on both localities, except in Gujrat, where Cr concentration (2.02±0.12 mg/L) was recorded slightly higher in livers of males.

Table 2 shows analysis of variance (ANOVA) of nickel and chromium concentrations among muscles, gender, location and metals. There was significant ($P<0.05$) difference between muscles, location and metals, while non-significant difference between gender. Furthermore, there was interaction effect between gender × location, gender × metal and location × metal. Whereas, there was no interaction effect between muscle × gender, muscle × location and muscle × metal

Both muscles and liver samples from Kotla showed positive correlation, while samples from Gujarat were negatively correlated. At Kotla, Ni and Cr concentration

Table 1. Analysis of nickel and total chromium concentrations mg/ L wet wt in black kite tissues of Kotla Arab Ali Khan and Gujrat, Pakistan in 2013

Biological material	Gender	n	Ni		Cr	
			Kotla Mean±SD	Gujrat Mean±SD	Kotla Mean±SD	Gujrat Mean±SD
Muscle	Both sexes	12	37.43±5.90	38.70±2.10	1.90±0.61	1.74±0.56
	Male	06	36.12±5.84	37.83±2.10	1.76±0.87	1.93±0.14
	Female	06	38.75±6.18	39.32±2.01	2.04±0.07	1.58±0.70
Liver	Both sexes	12	35.18±5.53	35.07±4.72	1.91±0.58	1.67±0.51
	Male	05	34.67±6.22	33.42±6.19	1.70±0.80	2.02±0.12
	Female	07	35.69±5.29	36.25±3.31	2.12±0.07	1.41±0.54

Table 2. Analysis of variance (ANOVA) of nickel and chromium concentrations among muscles, gender, location and metals, Pakistan in 2013

Source	df	MS	F	P
Muscle	1	0.013	7.39	0.008
Gender	1	0.000061	0.33	0.566
Location	1	0.0072	3.96	0.049
Metal	1	41.10	22350.9	0.000
Muscle × Gender	1	0.000026	0.01	0.905
Muscle × Location	1	0.000096	0.52	0.471
Muscle × Metal	1	0.004	1.95	0.166
Gender × Location	1	0.068	37.36	0.000
Gender × Metal	1	0.008	4.41	0.038
Location × Metal	1	0.011	5.89	0.017
Error	85	0.0018	-	-

was positive but non significant (R^2 = 0.324; P = 0.304) correlation was observed in black kite muscle, while Ni and Cr concentrations of liver samples were significant (R^2 = 0.599; P = 0.040). At Gujrat, negative correlations were recorded for Ni and Cr in muscle (R^2 = -0.326) and liver (R^2 = -0.271) (Table 3) but both were non significant (P = 0.301 & P = 0.393).

Table 3. Paired samples correlation between metals concentrations of liver and keel muscles in black kites *(Milvus migrans)* at Kotla Arab Ali Khan and Gujrat, Pakistan 2013

Sites	Correlated metals	Biological material	n	R^2	P-value
Kotla	Ni vs Cr	Muscle	12	0.324	0.304
	Ni vs Cr	Liver	12	0.599	0.040
Gujrat	Ni vs Cr	Muscle	12	-0.326	0.301
	Ni vs Cr	Liver	12	-0.271	0.393

There was almost fair balance between males (n = 11) and females (n = 13), furthermore, these 24 were categorized into adults (n = 14) and sub-adults (n = 10) of black kite collected from Kotla and Gujrat. This makes the capturing method quite random. The metal quantification from raptors tissues is considered a valuable method for prediction of their habitat's quality and contamination, and furthermore, it indicates the potential risk of exposure for birds and humans (Zaccaroni *et al.,* 2008; Fox, 2001; Rainbow and Blackmore, 2001; Movalli, 2000).

Outridge and Scheuhammer (1993) described level of metals in tissues of wild mammals and birds, and reported maximum value of Ni concentration in uncontaminated (~0.1 to 5 μg/g) and contaminated (-0.5 to 80 μg/g) on dry weight basis. In the present study, the Ni concentration 37.43±5.90 mg/L and 35.18±5.53 mg/L were recorded from muscles and livers respectively from Kotla, whereas in Gujrat 38.70±2.10 mg/L and 35.07±4.72 mg/L were, found in muscles and livers, respectively. It is higher than the concentrations documented by Zaccaroni *et al.* (2008) and Van Wyk *et al.* (2001). Van Wyk *et al.* (2001), documented Ni concentration (dry matter basis) in liver and muscles of three vultures *viz.,* white backed vulture *(Pseudogyps africanus)* contained 13.92 ± 4.64 μg/g and 10.75±4.37 μg/g, respectively, Lappet faced vulture *(Torgos tracheliotos)* contained 7.83 and 9.23 μg/g, respectively, whereas, Cape griffon *(Gyps coprotheres)* contained 11.40 μg/g in muscles and 12.82 μg/g in liver. Eisler (1998) reported Ni concentration in *Anas platyrhynchos* (0.1-1.4 mg/kg fresh wt.) in liver and breast muscles (0.1-0.8 mg/kg fresh wt.). *Phalocrocorax atriceps* muscles (0.29 mg/kg dry wt.), liver of *Bonasa umbellus* (1.0 mg/kg dry wt.), *Fulica Americana* from (1.5 mg/kg fresh wt.) in muscle, Gavia immer (1.1 mg/kg fresh wt.), *Larus fuscus* in liver (2.0 mg/ kg dry wt.) and in muscle (5.0 mg/kg dry wt.). This present work indicates alarming conditions with respect to nickel concentration in the studies areas. This alarming Ni concentration in the study area is due to electrical industry and vehicles exhaust etc. from where Ni is emitted (Khan *et al.,* 1990).

Chromium is an essential element for all animals, however its tissue concentrations (>4 mg/kg dry wt.) indicates alarming conditions (Outridge and Scheu-hammer, 1993). Eisler (1986) reported that electroplating and metal finishing industries, municipal treatment plants, tanneries, oil drilling operations, and cooling towers are major factors for elevation of Cr levels in soil, air, water, and biota. Korenekova *et al.* (2008) reported Ni (0.548 mg/kg) concentration in pheasants. In the present study, Cr concentration in muscles samples from Kotla and Gujrat was 1.90±0.61 mg/L and 1.74± 0.56 mg/L, respectively, while in liver it was 1.91±0.58 mg/L and 1.67±0.51 mg/L, respectively. Our findings are closely in line with the report of Eisler (1986), where *Somateria mollissima* and *L. fuscus* muscle, liver, kidney, and egg contained Cr concentration (< 1.0 mg/kg dry wt.), *Aythya valisineria* constituted 0.02 mg/kg fresh wt. Cr concentration in liver, *Pandion haliaetus* and *Pelecanus occidentalis,* with concentration of chromium in liver of 0.2 mg/kg fresh wt. Furthermore, he reported that Cr concentration greater than 4.0 mg/kg dry wt., indicated Cr contamination. In the present study, Cr concentration was recorded lesser than the toxic level (<4 mg/kg dry wt.).

Conclusion

The present work revealed that black kite can be used as a biomonitor of heavy metals contaminated environment. Ni and Cr concentration not only varies in sex groups but also in different age groups in black kite. The present study evaluated metals concentrations in small samples of black kite (in limited areas); it would be desirable to better understand metal residues in kite populations from uncontaminated reference areas as well as contaminated areas, especially metal doses to the embryos during nesting.

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