

Estimation of heavy metal residues from the feathers of Falconidae, Accipitridae, and Strigidae in Punjab, Pakistan

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Abstract: The concentrations of heavy metals (Cd, Zn, Ni, Pb, Cu, As) in feather samples of raptors of 3 families were determined from southern, central, and northern parts of Punjab Province. Deposition of metals significantly varied among the families for Cd, Zn, Ni, and Pb; further, these 4 metals also significantly differed between Accipitridae and Falconidae, and between Falconidae and Strigidae. Similarly, Cd, Ni, and As were significantly different among southern, central, and northern Punjab. Falconidae deposited higher concentrations of metals than Accipitridae and Strigidae, with the Common Kestrel being more susceptible. Likewise, central Punjab appeared to be more polluted than northern and southern Punjab. The concentrations of metals reported are alarming and require proper management.

Key words: Heavy metals, raptors, feathers, Punjab

1. Introduction

Heavy metals have always been natural components of our environment present in very low concentrations and are continually released into the environment from natural sources like volcanic activity or weathering of rocks. Moreover, industrial processes and some agricultural activities have greatly increased the mobilization of many metals in freshwater ecosystems. Therefore, heavy metal pollution has become an important problem in recent years. These metals are potentially harmful to most organisms at some level of exposure and absorption (Güven et al., 1999). Heavy metals, to a large extent, are dispersed in the environment through industrial effluents, organic wastes, refuse, burning, transport, and power generation (Agarwal, 2002). The manufacture of pigments, drugs, agrochemicals, plastics, and batteries; electroplating; and discharge of untreated effluents from different industries cause pollution (Raihan et al., 1995). Metals like mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), cobalt (Co), and zinc (Zn) are highly toxic to both flora and fauna components of the ecosystem (Burger and Gochfeld, 1997; Gerbersmann et al., 1997; Lee et al., 2006).

Heavy metals have a serious impact on the environment and can threaten the ecosystem's stability (Güven et al., 1999; Sanpera et al., 2000; Battaglia et al., 2003). Pb and

Cd are nonessential heavy metals for organisms and are distributed globally from burning of fossil fuels, road traffic, and industries (Kenntner et al., 2003). Cd is listed in the "most dangerous trace element category" and is present in both the environment and food with long persistence and high toxicity, while the toxic effect of Pb has been well established in birds for over a century (Battaglia et al., 2003). Dietary exposure to high levels of Pb and Cd can be toxic to avian species (Scheuhammer, 1987) and influence the endocrine system (Stoica et al., 2000; Martin et al., 2003), kidneys (Nordberg, 1971), reproduction (Buerger et al., 1986), behavioral response (Furness, 1996; Hui, 2002), molting, migration (Cheney et al., 1981; Honda et al., 1986), enzymes involved in hemoglobin formation, and growth rates (Eisler, 1988). Ni, which is used in metal plating, alloy making, as a catalyst in chemical reactors, and in battery making, is deposited in rivers and streams through wastes and is taken up by animals and birds, affecting their respiratory system, causing asthma, as well as birth defects, vomiting, and damage to DNA. Zn is used in alloying, dyeing, and manufacturing electric goods, insecticides, and cosmetics (Irwin, 1997), and causes reduced species diversity and abundance, as well as fainting, nausea, and stomach disorders (Peplow, 2000; Perez-Lopez et al., 2008). Cu is

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an essential trace element that is metabolically regulated, but its excesses result in additional stress to birds already living in stressful environmental conditions (Debacker et al., 2000). As is a natural metalloid present in both organic as well as inorganic forms in soil, air, and water (Huang et al., 2004; Duker et al., 2005), and it causes portal cirrhosis, anemia, sensory disturbance, liver damage, different cancers, genetic disorders, and weight loss (Webb, 1966; Nemery, 1990; Zhao et al., 1997; Abernathy et al., 1999; Huan et al., 2004; Duker et al., 2005).

Heavy metals have been well studied in the aquatic food chain (Burger and Gochfeld, 1993; Burger and Gibbons, 1998; Güven et al., 1999; Kim and Koo, 2008; Taggart et al., 2009) and result in poor breeding as well as high mortality for sea eagles (*Haliaeetus* spp.) and ospreys (*Pandion haliaetus*). The terrestrial food chains are relatively less studied (Pain et al., 1995; Movalli, 2000; Dauwe et al., 2003; Kenntner et al., 2007; Zolfaghari et al., 2007; Perez-Lopez, 2009); however, due to environmental pollutants population decline has also been reported for raptors (Palma et al., 2005).

Public interest in environmental contamination has increased in recent years and therefore monitoring, evaluation, management, and remediation of ecological damage are required (Kushlan, 1993; Grove et al., 2009). In the ecosystem, assessment of change in all the living components, properties, or functions is impossible; however, certain biological components like birds can act as bio-indicators for some conditions (Movalli, 2000). Among the species, toxicity and heavy metal exposure in birds are relatively well studied (Muralidharan et al., 2004). Since the 1960s, the role of birds as monitors of environmental pollution has been recognized, because birds are more sensitive to human-induced environmental changes (Denneman and Douben, 1993).

Generally, birds acquire heavy metals through intake of food, drinking, and geophagy. The rate of heavy metal absorption varies depending on species physiology, metal properties, and bio-availability in the environment. After absorption, metals circulate in the body, are excreted or get deposited in various body tissues, or are sequestered in feathers (Furness et al., 1986). The high toxicity of heavy metals may result in eggshell thinning, low reproduction rate, suppression of the immune system, reduced growth/weight, and developmental malformations, and all these lead to possible population decline in birds (Spahn and Sherry, 1999; Dauwe et al., 2006). Long-term exposure to heavy metals can also cause disruptive behavior and reduction in disease resistance and affect other physiological processes (Dauwe et al., 2005).

It is very difficult and risky to capture a protected and rare raptor species for sample collection. Noninvasive methods like regurgitated pellets or blood samples can

be useful for determining heavy metal exposure, its seasonality, and food items with level of heavy metal load (Mateo et al., 1999). However, these methods can still be difficult sometimes for raptors inhabiting mountainous or wooded habitats in the nonbreeding season. Alternately, collecting feathers of raptors as well as water birds allows the determination of temporal and spatial trends of heavy metals in birds without any damage and therefore is a more suitable strategy for threatened or endangered species (Burger, 1993).

Heavy metal pollution has increased in recent years in South Asia (Hardoy et al., 1992). In Pakistan, herbicides, pesticides, agricultural uses of fertilizers, and industrial wastes are the main sources of heavy metal contamination (Baluch, 1995). Heavy metals such as Hg have been reported in higher than normal concentrations from the sea and rivers of Pakistan (Tariq et al., 1994; Tehseen et al., 1994). The present study aimed to determine the heavy metal exposure of different raptor species from central, southern, and northern parts of Punjab (Pakistan) through feather samples analysis.

2. Materials and methods

2.1. Study area

Punjab Province is mainly a fertile plain with rivers and irrigation canals. It also includes deserts like Thal and Cholistan in the southern parts of the province. The River Indus and its tributaries traverse Punjab from north to south. The landscape is notably irrigated and canals can be found over a major part of the province. Weather extremes are notable, from the hot and barren south to the cool hills of the north. The northern parts of the province comprise Potohar Plateau, which is an arid zone. The foothills of the Himalayas are found in the extreme north (Figure 1).

For the current study, feather samples ($n = 45$) were collected from 14 districts of Punjab Province, namely Lahore, Gujranwala, Okara, Kasur, and Jhang (central Punjab); Rawalpindi, Jhelum, Chakwal, Mianwali, and Khushab (northern Punjab); and Rajanpur, Bahawalnagar, D.G.Khan, and Rahim Yar Khan (southern Punjab; Figure 1; Table 1) Samples from Nagarparkar and Kashmore (Sindh Province; adjacent to southern Punjab) were included in the southern Punjab region, while those from Islamabad (adjacent to northern Punjab) were considered in northern Punjab.

2.2. Sampling

Birds of prey are mostly protected; hence, noninvasive techniques are required to collect the samples. Feathers can easily be collected and in numerous studies have been used to determine heavy metal levels (Burger and Gochfeld, 1993; Hahn et al., 1993; Dauwe et al., 2002, 2003; Zolfaghari et al., 2007; Kim and Koo, 2008; Burger et al., 2009). The specimens of birds belonging to 3 main

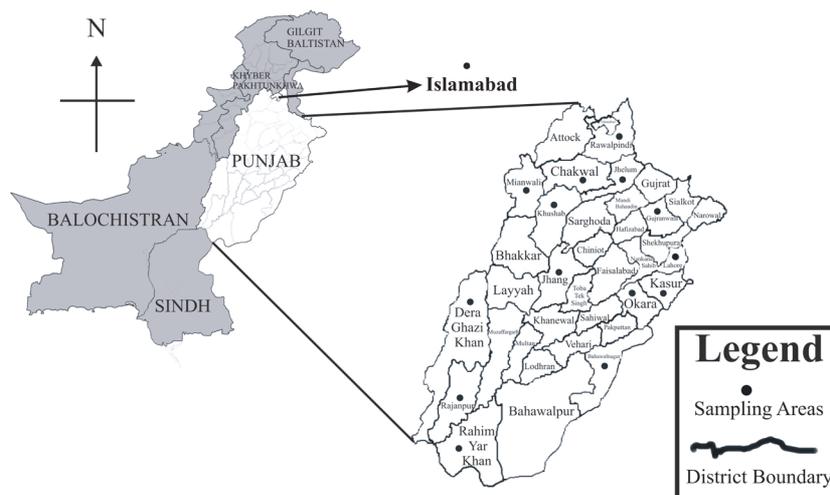


Figure 1. Map of Pakistan/Punjab showing the study districts.

families (Accipitridae, Falconidae, and Strigidae) were collected from different sites within the province (Table 1; Figure 1) as per availability of the birds during the study period (May 2008–May 2009). Trained falcon trappers were approached for the collection of feather samples. Only 3 to 4 breast feathers were plucked from each adult bird; they were placed in sealed plastic bags with an ID number. This consistency in sample collection only from the breast is important because variations in metal levels may exist among feathers from different locations on the body of individual birds (Bortolotti and Barlow, 1985). It is also suggested that body feathers are more representative of metal levels than flight feathers (Doi and Fukuyana, 1983; Furness et al., 1986). These breast feathers grow back rapidly and their removal does not affect the bird's flight or thermoregulatory efficiency (Movalli, 2000). Difficulties experienced in trapping the birds and collecting their feathers affected the total sample size. To overcome this problem, samples were pooled familywise (Falconidae, Strigidae, and Accipitridae), and regionwise (northern Punjab, central Punjab, and southern Punjab) for comparison.

2.3. Analysis

Feather samples were processed for atomic absorption spectrophotometry following Fransion (1981), for determination of various metal concentrations in the feather samples. Firstly, feather samples were washed 3 times with tap water, rinsed with distilled water, and then washed in acetone following Saeki et al. (2000) and Battaglia et al. (2003) to get rid of the external pollutants (Goede and Bruin, 1984). The samples were then dried at 80 °C for 12 h in an oven, following Saeki et al. (2000). Finally they were cut into small pieces, weighed, and transferred into quartz crucibles. About 1.0 mL of HNO₃

and 0.25 mL of perchloric acid were added to the crucibles, which were covered with watch glasses. The digestion was carried out by using a hot plate with a magnetic stirrer (HB 502 Bibby), initially at low temperature and then at higher temperature. The digested samples were analyzed in triplicate for heavy metal concentrations using an atomic absorption spectrophotometer (G.B.C. 932 Plus, UK). Certified standard solutions of the elements (1000 µg/g) were used for the preparation of the elements' working standard solutions.

SPSS (version 18.0) was used for the statistical analysis. The distribution of metal concentrations was not normal (Shapiro–Wilk test, $P < 0.05$); therefore, we applied the nonparametric statistic. Kruskal–Wallis one-way analysis of variance was used to determine the differences in metal concentrations among the regions and families. When a difference was found, a posteriori pairwise comparisons were made using the Mann–Whitney U test.

3. Results

Feather samples of 45 birds from 17 species of raptors belonging to the families Accipitridae, Falconidae, and Strigidae were collected. Mean concentrations of various metals in the feather samples of the different raptor species are given in Table 2. The comparison of the mean values of metals showed that maximum metal concentration was Ni (500 µg/g) deposited by the Common Kestrel (*Falco tinnunculus*) collected from the border area of southern Punjab adjacent to Kashmir. Overall, the highest mean values for metal levels in all the raptors were found for Zn, followed by Ni.

3.1. Family variations

In the family Accipitridae, a total of 26 different samples of feathers from 9 species were analyzed for metal

Table 1. Details of the study districts of southern, central, and northern Punjab Province, which were sampled for the collection of feathers of raptors.

Name of district	Coordinates	Average elevation (meters above sea level)	No. of samples collected
Southern Punjab			
Rajanpur	N29°6'13.76" E70°19'12.77"	102	3
Rahim Yar Khan	N28°24'59.10" E70°17'59.10"	88	5
Dera Ghazi Khan	N30°2'59.99" E70°37'60.00"	138	1
Bahawalnagar	N29°59'48.88" E73°15'4.20"	172	1
Okara	N30°48'4.97" E73°26'54.00"	199	2
Total			12
Central Punjab			
Lahore	N31°32'36.47" E74°20'18.66"	236	7
Kasur	N31°7'3.40" E74°26'59.82"	230	2
Gujranwala	N32°8'60.00" E74°10'60.00"	246	2
Jhang	N31°15'48.28" E72°20'34.63"	170	1
Khushab	N32°17'24.96" E72°20'55.83"	202	2
Mianwali	N32°35'6.20" E71°32'38.40"	232	3
Total			17
Northern Punjab			
Jhelum	N32°56'0.63" E73°43'14.57"	253	7
Chakwal	N33°40'38.00" E72°51'21.00"	567	1
Rawalpindi	N33°8'60.00" E73°30'35.78"	373	8
Total			16
Grand total			45

Table 2. Mean concentrations ($\mu\text{g/g}$) of different metals estimated from the feather samples of raptor species of 3 different families, collected during the current study from various regions of Punjab Province. (ND: Not detectable; NA: Not analyzed)

Bird family	Name of bird species	Sample size	Metal concentrations ($\mu\text{g/g}$)					
			Cd	Zn	Ni	Pb	Cu	As
Accipitridae	Steppe Eagle (<i>Aquila nipalensis</i>)	7	0.518	62.53	21.82	7.75	12.38	4.74
	Black Kite (<i>Milvus migrans</i>)	6	0.85	104.8	32.61	7.34	14.08	9.46
	Black Shouldered Kite (<i>Elanus caeruleus</i>)	3	1.472	186.9	96.89	49.34	12.95	11.01
	Common Buzzard (<i>Buteo buteo</i>)	1	0.67	90.57	4.96	1.74	18.59	NA
	White Eyed Buzzard (<i>Butastur teesa</i>)	1	2.6	59.54	ND	17.95	ND	13.82
	Marsh Harrier (<i>Circus aeruginosus</i>)	1	0.72	94.09	52.5	4.75	19.32	6.52
	Eurasian Sparrow Hawk (<i>Accipiter nisus</i>)	3	11.96	143.9	83.89	38.83	28.25	12.95
	Red Headed Vulture (<i>Sarcogyps calvus</i>)	3	0.33	48.49	3.2	5.88	6.28	NA
	Shikra (<i>Accipiter badius</i>)	1	3.2	172.7	140	42	4.66	28
Falconidae	Peregrine Falcon (<i>Falco peregrinus</i>)	7	3.09	165.6	113.5	24.43	27.56	12.97
	Saker Falcon (<i>Falco cherrug</i>)	1	4	294.3	152.9	ND	10	3.24
	Common Kestrel (<i>Falco tinnunculus</i>)	1	5.2	289.3	500	62.66	ND	44.55
	Laggar Falcon (<i>Falco jugger</i>)	2	3.34	154.6	264.2	32.23	ND	39.34
	Red Headed Merlin (<i>Falco chicquera</i>)	1	1.07	125.7	117.5	18.69	22.22	18
Strigidae	Short Eared Owl (<i>Asio flammeus</i>)	1	1.7	126.3	95.75	12	8.75	4.81
	Spotted Owlet (<i>Athene brama</i>)	4	1.28	90.74	68.44	6.08	7.61	6.44
	Eagle Owl (<i>Bubo bubo</i>)	1	0.07	ND	4.01	17.9	3.38	1.06
	Himalyan Barred Owlet (<i>Glaucidium cuculoides</i>)	1	0.86	56.64	68.22	8.57	4.11	4.68

concentrations. Zn was found to be the most heavily deposited metal, with a mean concentration of $119.14 \pm 15.99 \mu\text{g/g}$, followed by Ni ($47.73 \pm 9.16 \mu\text{g/g}$) (Table 3). Mean metal concentrations in the family Accipitridae ranged from $2.19 \mu\text{g/g}$ for Cd to $119.14 \mu\text{g/g}$ for Zn (Table 3).

For the family Falconidae, a total of 12 feather samples were collected from 5 different species. The maximum mean concentration was found for Zn ($181.46 \pm 18.73 \mu\text{g/g}$), with Ni being second ($166.65 \pm 35.75 \mu\text{g/g}$). The lowest concentration ($3.22 \pm 0.38 \mu\text{g/g}$) deposited was found for Cd, whereas Pb, Cu, and As were deposited in moderate concentrations (Table 3). In the family Falconidae, variation in metal deposition in the feathers was noticeable. In the Peregrine Falcon (*Falco peregrinus*), Saker Falcon (*Falco cherrug*), and Red-headed Merlin (*Falco chicquera*), the concentrations of Zn were $165.6 \mu\text{g/g}$, $294.3 \mu\text{g/g}$, and $125.7 \mu\text{g/g}$, respectively, whereas in the Common Kestrel and Laggar Falcon (*Falco jugger*), Ni was the most heavily deposited metal ($500 \mu\text{g/g}$ and $264.2 \mu\text{g/g}$, respectively). In all 5 species of falcons analyzed, Cd showed the lowest concentrations (Table 2).

In the family Strigidae, a total of 7 feather samples of 4 species were analyzed. Zn was the most heavily deposited metal, with a mean concentration of $90.98 \pm 28.06 \mu\text{g/g}$, followed by Ni ($73.63 \pm 29.38 \mu\text{g/g}$). Cd ($1.11 \pm 0.36 \mu\text{g/g}$) was again the least deposited metal of all the 6 (Table 3). Among the various species of the family Strigidae, the Short-eared Owl (*Asio flammeus*) had the highest concentration of metal (Zn = $126.3 \mu\text{g/g}$) in the feather samples, followed by the Spotted Owllet (*Athene brama*) showing a concentration of $90.74 \mu\text{g/g}$ for Zn. In all the species of this family, Cd showed a relatively low deposition (Table 2).

3.2. Comparisons among families

The Kruskal–Wallis test showed a significant difference (Tables 3 and 4) among the 3 families for Cd, Zn, Ni, and Pb ($P < 0.05$). A significant difference was found between the families Accipitridae and Falconidae for Cd ($U = 31.0$, $P < 0.01$), Zn ($U = 67.5$, $P < 0.01$), Ni ($U = 30.0$, $P < 0.01$), and Pb ($U = 79.5$, $P < 0.05$; Mann–Whitney U test). Similarly, the families Falconidae and Strigidae also differed significantly for Cd ($U = 8.0$, $P < 0.01$), Zn ($U = 10.0$, $P < 0.01$), Ni ($U = 17.0$, $P < 0.05$), and Pb ($U = 11.0$, $P < 0.01$; Mann–Whitney U test). However, concentrations of all metals were nonsignificantly different between the families Accipitridae and Strigidae ($P < 0.05$). Falconidae deposited the highest concentrations of metals, followed by Accipitridae and then Strigidae.

3.3. Regional variations

The southern Punjab region showed that the mean concentration of Ni was highest deposited ($153.4 \pm 57.26 \mu\text{g/g}$), followed by Zn ($122.17 \pm 4.09 \mu\text{g/g}$), while Cd was the least deposited ($1.83 \pm 0.49 \mu\text{g/g}$) metal. Pb, Cu, and As showed moderate concentrations (Figure 2). The central Punjab region showed that Zn was deposited in the highest mean concentrations ($127.1 \pm 16.76 \mu\text{g/g}$) of all the metals studied, followed by Ni ($90.39 \pm 15.08 \mu\text{g/g}$), while Cd showed the lowest ($3.35 \pm 1.64 \mu\text{g/g}$) mean concentrations (Figure 2). Likewise, northern Punjab also showed Zn being the most heavily deposited metal in the feather samples ($158.58 \pm 26.42 \mu\text{g/g}$), followed by Ni ($42.48 \pm 11.74 \mu\text{g/g}$), whereas Cd was the least ($1.14 \pm 0.36 \mu\text{g/g}$) deposited (Figure 2).

3.4. Comparisons among regions

Pooled data of each region showed a significant difference in the concentrations of Cd, Ni, and As ($P < 0.05$, Kruskal–

Table 3. Pooled concentrations of various heavy metals ($\mu\text{g/g}$) estimated from the feather samples of raptors belonging to 3 different families. Values are expressed as mean \pm SE. n = sample size.

Metals	Raptor families		
	Accipitridae (n = 26)	Falconidae (n = 12)	Strigidae (n = 7)
Cd	2.19 ± 1.28^a	3.22 ± 0.38^b	1.11 ± 0.36^c
Zn	119.14 ± 15.99^a	181.46 ± 18.73^b	90.98 ± 28.06^c
Ni	47.73 ± 9.16^a	166.65 ± 35.75^b	73.63 ± 29.38^c
Pb	17.88 ± 4.98^a	28.81 ± 4.51^b	12.57 ± 1.79^c
Cu	17.64 ± 4.05^a	32.17 ± 13.66^a	7.78 ± 2.48^a
As	11.07 ± 1.89^a	19.61 ± 4.77^a	5.19 ± 1.56^a

Same superscript in each row shows nonsignificant difference ($P < 0.05$); Mann–Whitney U test.

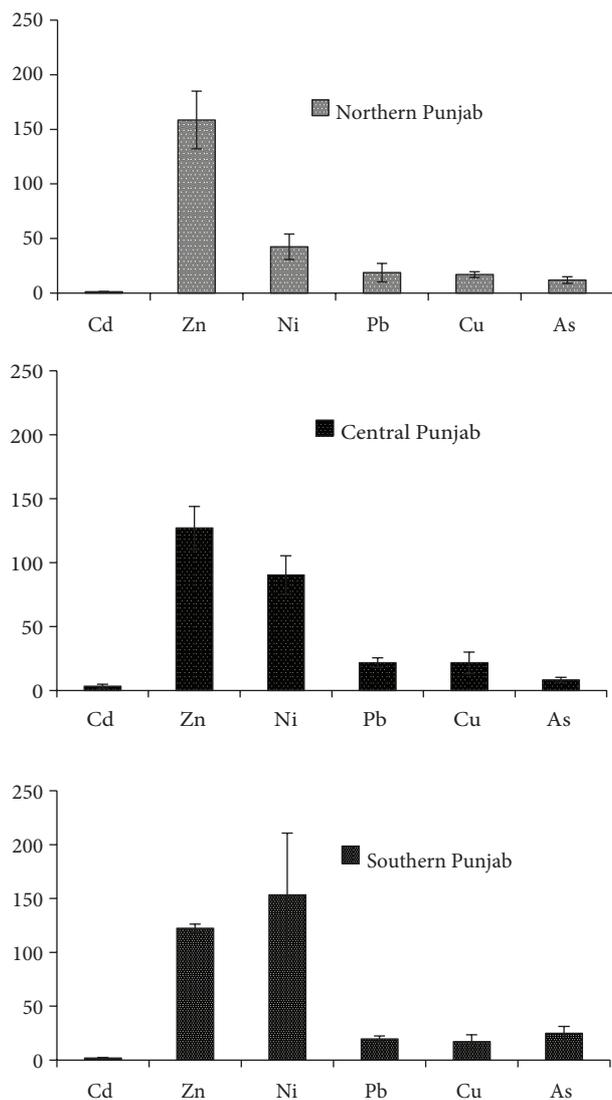


Figure 2. A comparison of concentrations ($\mu\text{g/g}$) of 6 different metals estimated from the feathers of raptors in 3 different regions of Punjab Province. Values expressed as mean \pm SE.

Wallis test) among the feather samples of raptors obtained from the 3 regions (Table 4). As significantly differed ($U = 17.0$, $P < 0.01$; Mann-Whitney U test) between southern and central Punjab but metal concentrations of central and northern Punjab were nonsignificantly different. Southern and northern Punjab appeared significantly different for Cd ($U = 33.0$, $P < 0.01$), Ni ($U = 33.0$, $P < 0.01$), Pb ($U = 39.0$, $P < 0.05$), and As ($U = 26.0$, $P < 0.01$; Mann-Whitney U test). Among the families of each region, only Accipitridae was significantly different ($U = 38.0$, $P < 0.05$) in southern and central Punjab, while the other families varied in mean values among the regions but nonsignificantly (Table 5). Central Punjab appeared to be most polluted, followed by northern and then southern Punjab.

4. Discussion

Environmental pollution is one of the most devastating ecological crises facing the world today. It is especially true for most developing countries in South Asia, such as Nepal, India, Bangladesh, and Pakistan (Karn and Harada, 2001), where water pollution is more severe and critical near urban areas due to huge amounts of pollution load discharged by urban activities. About 90% to 95% of all domestic sewage and 75% of all industrial effluent are discharged into surface waters without any treatment (Hinrichsen et al., 1997; Pandey, 2006).

Pakistan is an agricultural country; however, population increase, unplanned urbanization, deforestation, and rapid industrialization over the past few decades have all contributed to environmental pollution. The main sources of environmental pollution in the country are sewage water, city refuse, fertilizers, pesticides, automobiles, industrial effluents, and leather tanning (Hanif et al., 1987; Tahir et al., 1998; Aftab et al., 2000). Huge amounts of solid sludge and effluent water containing acids, alkalis, sodium chloride, and heavy metals are released daily in tons into nullahs (seasonal water courses) and streams; this waste directly flows into rivers and eventually into the ocean (Zaman and Ara, 2000; Tahir and Naseem, 2007). In Pakistan, dumping of industrial waste, effluents, and sewage into the soils and wetlands is a common activity that ultimately affects the food chain and the ecosystem. As a result, soils, air, and water are heavily polluted with various heavy metals like Hg, As, Pb, Zn, Mn, Li, Cd, and Cr (Abbas et al., 2004).

Raptors are at a high trophic level; many of them are territorial and nonmigratory with extended home ranges and long life spans. Hence their risk of accumulating metals is higher and these may accumulate in soft body tissues, bones, feathers, and eggs. Raptor species are thought to be more vulnerable to metals (Stout and Trust, 2002) and are important as bio-indicators (Wayland and Bollinger, 1999; Zaccaroni et al., 2003). Monitoring of heavy metal levels in feather samples of raptors and water birds allows researchers to determine the spatial and temporal trends without sacrificing the birds, especially threatened and endangered species (Burger, 1993).

Given the increased concern about heavy metal contamination, it is very important to measure metal concentrations in wild species. Raptors are one of the better choices in this context. In the current study, feather samples of raptors were collected from the southern, central, and northern regions of Punjab Province to compare the metal contamination in the 3 regions. The southern Punjab is a cotton belt and the central Punjab is heavily industrialized, whereas the northern region of the province comprises scantily industrialized units. In the current study, Cd, Cu, and Pb were significantly higher

Table 4. P-values of Kruskal–Wallis test for 3 families of raptors and 3 different regions of the Punjab.

Metals	Families			Regions		
	Chi-square	df	P-value	Chi-square	Df	P-value
Cd	15.419	2	0.000	6.184	2	0.045
Zn	8.970	2	0.011	2.207	2	0.332
Ni	13.775	2	0.001	6.696	2	0.035
Pb	6.906	2	0.032	4.979	2	0.083
Cu	0.343	2	0.842	1.591	2	0.451
As	5.078	2	0.079	13.540	2	0.001

Table 5. Comparison of mean metal values ($\mu\text{g/g}$) among 3 families in 3 regions of Punjab, Pakistan.

Metals	Northern Punjab (n = 16)			Southern Punjab (n = 12)			Central Punjab (n = 17)		
	<i>Accipitridae</i>	<i>Falconidae</i>	<i>Strigidae</i>	<i>Accipitridae</i>	<i>Falconidae</i>	<i>Strigidae</i>	<i>Accipitridae</i>	<i>Falconidae</i>	<i>Strigidae</i>
Cd	0.82 ± 0.21	4.04 ± 0.88	1.28 ± 0.56	0.86 ± 0.44	2.63 ± 0.66	-	5.90 ± 4.70	3.69 ± 0.17	0.88 ± 0.47
Zn	768.06 ± 667.66	163.63 ± 56.36	90.75 ± 42.04	59.82 ± 15.94	174.11 ± 27.76	-	116.5 ± 22.04	201.53 ± 34.65	60.96 ± 36.51
Ni	33.58 ± 9.99	120.59 ± 7.26	68.44 ± 46.40	12.42 ± 10.18	194.21 ± 68.98	-	79.28 ± 18.18	148.34 ± 36.14	55.99 ± 27.18
Pb	16.30 ± 7.79	32.89 ± 8.52	6.09 ± 3.73	8.06 ± 3.60	24.05 ± 7.64	-	28.06 ± 7.64	19.18 ± 7.97	12.83 ± 2.72
Cu	12.76 ± 2.86	6.67 ± 6.66	7.61 ± 4.20	7.63 ± 3.52	13.36 ± 7.25	-	12.77 ± 10.87	32.91 ± 25.67	5.59 ± 1.58
As	6.19 ± 1.60	18.88 ± 17.54	6.43 ± 2.55	4.06 ± 2.74	29.50 ± 5.40	-	14.41 ± 4.50	5.13 ± 2.55	4.72 ± 2.69

in central Punjab than in northern and southern Punjab, while deposition of As and Ni was significantly greater in southern Punjab than in the other 2 regions. The central region is densely populated, with a heavy traffic load, and is more industrialized as compared to the other 2 regions; therefore, it is more polluted than the other 2 regions and safe disposal of industries wastes is required.

Three studies regarding heavy metal deposition in birds from various regions of Pakistan have been conducted so far (Table 6). Movalli (2000) studied metal deposition (Cd, Ni, Zn, and Pb) in feathers of the Lagger Falcon from various regions of southern Punjab and Sind provinces. The concentrations of Cd, Zn, Ni, and Pb in the feather samples of Bahawalnagar and Bahawalpur (south Punjab) were all much lower than those estimated in the current study for all 3 families and regions of the Punjab. This indicates that for the last 10 years the levels of these metals have increased considerably in the environment, especially in this part of the country. Estimates of Cd concentrations in feathers of Little Egrets and Cattle Egrets by Boncompagni et al. (2003) from Lake Haleiji (0.09 $\mu\text{g/g}$), Karachi (0.10 $\mu\text{g/g}$), and Taunsa Barrage (0.06 $\mu\text{g/g}$) were lower than the levels of metals obtained in the

current study for southern (1.83 $\mu\text{g/g}$), central (3.35 $\mu\text{g/g}$), and northern (1.14 $\mu\text{g/g}$) areas, indicating a greater load of heavy metal pollution in recent times. Conversely, Malik and Zeb (2009) reported greater accumulation of Cd, Zn, and Pb and lower levels of Ni and Cu in the feather samples of Cattle Egrets from Chenab and Ravi rivers (central Punjab) and Lake Rawal, Islamabad (northern Punjab) as compared to the results of the current study. Similarly, the levels of As in the current study were much higher than those estimated by Boncompagni et al. (2003) from Karachi and southern Punjab. The trophic level of egrets is, however, different from that of raptors and perhaps this comparison is not very suitable but still it provides some information regarding the greater levels of metals.

The concentrations of metals in feather samples of the 3 raptor families in the current study have shown that the family Falconidae had significantly higher levels of Cd, Zn, Ni, and Pb than Accipitridae and Strigidae; the Common Kestrel showed the maximum average metal deposition of Ni (500 $\mu\text{g/g}$) and Zn (289.3 $\mu\text{g/g}$) among all the raptor samples analyzed. The Lagger Falcon also showed heavy metal deposition (264.2 $\mu\text{g/g}$ and 154.6 $\mu\text{g/g}$ for Ni and Zn, respectively), ranking second after the Common Kestrel.

Table 6. Comparison of mean concentrations of heavy metals ($\mu\text{g/g}$) in feathers of raptors in the present study (collected from 3 different regions of Punjab Province) with those in published records regarding Pakistan for the previous 10 years.

Authors	Study area	Bird species	Cd	Zn	Ni	Pb	Cu	As
Current study	Southern Punjab, Pakistan	Raptors	1.83	122.2	153.4	19.52	16.92	24.67
	Central Punjab, Pakistan	Raptors	3.35	127.1	90.39	21.73	21.59	8.24
	Northern Punjab, Pakistan	Raptors	1.14	158.59	42.48	18.67	16.88	11.97
Malik and Zeb (2009)	River Chenab, Pakistan	Cattle Egret	3.1	133.8	9.0	37.5	4.0	-
	River Ravi, Pakistan	Cattle Egret	2.4	155.2	8.1	76.5	3.7	-
	Rawal Lake Reservoir, Pakistan	Cattle Egret	2.7	138.4	7.8	60.2	4.0	-
Boncompagni et al. (2003)	Lake Haleji, Pakistan	Little Egret	0.09	154.0		0.41		
		Intermediate Egret		214.0	-	0.41	-	-
	Karachi, Pakistan	Little Egret	0.10	199.7	-	4.62	-	0.48
	Taunsa, Pakistan	Little Egret		0.4	-	-	-	0.10
		Cattle Egret	0.06	69.3	-	0.73	-	0.10
Movalli (2000)	Bahawalnagar, Pakistan	Laggar Falcon	0.12	104.13	104.0	1.08	-	-
	Bahawalpur, Pakistan	Laggar Falcon	0.08	113.88	113.0	2.76	-	-
	Chachero, Pakistan	Laggar Falcon	0.10	0.00	0.0	1.09	-	-
	Jacobabad, Pakistan	Laggar Falcon	0.11	0.00	0.0	1.28	-	-
	Karachi, Pakistan	Laggar Falcon	0.0	104.00	104.0	0.0	-	-
	Mithi, Pakistan	Laggar Falcon	0.11	111.33	111.3	0.93	-	-

This shows that falcons have a higher ability to accumulate more metals from the environment through the food chain. Since some of these metals, such as Cd, Ni, and Pb, were found in higher concentrations in the species of this family, falcons are expected to be more vulnerable to metal pollution. According to Palma et al. (2005), population declines were attributed to environmental contaminants for some raptor species feeding on terrestrial food chains. It was reported previously that heavy metals can reduce reproductive success and in severe cases even cause death (Sanpera et al., 2000); therefore, they are a serious threat to the survival of birds (Hernández et al., 1999). Movalli (2000) also documented higher metal deposition such as Cd, Zn, Pb, and Ni in the feathers of Laggar Falcons in southern Punjab (Bahawalnagar and Bahawalpur), indicating perhaps that falcons are more susceptible to metals.

The threshold level of Cd is considered above 3 ppm (dw) in the liver and indicates increased environmental pollution (Scheuhammer, 1987; Burgat, 1990). We found the mean concentration of Cd to be below the threshold level in Accipitridae and Strigidae but in Falconidae it was slightly higher (3.22 $\mu\text{g/g}$). It is accepted generally

that threshold limits for Zn do not exist (Taggart et al., 2006); however, Gasaway and Buss (1972) reported that the threshold level of Zn in birds is 1200 $\mu\text{g/g}$. In mallards, the clinical signs of Zn poisoning were observed at concentrations of 473–1990 ppm (dw) in the liver (Levengood et al., 1999), in waterfowls of 280–2900 ppm (Sileo et al., 2003), and in cage and aviary birds of 320–534 ppm (Doneley, 1992). In the present study, the Zn concentrations were lower than the threshold limits in all families. Pb concentrations below 2 ppm in the liver could be safe and this level could be considered the threshold level (Pain et al., 1995). A concentration of 30 ppm in hepatic tissue is considered a potentially lethal level for birds and of up to 6 ppm in raptors (Wayland et al., 1999; Wayland and Bollinger, 1999). We found mean concentrations of Pb from 12.57 $\mu\text{g/g}$ in Strigidae to 28.81 $\mu\text{g/g}$ in Falconidae, which could be dangerous. Wiemeyer et al. (1980) found 4 out of 18 ospreys (*Pandion haliaetus*) dead in the eastern USA with As concentrations higher than 5 ppm (dw), while 1 bird had an As hepatic level of 56 ppm (dw), enough for the death of this bird. In our study, mean concentrations of As were 11.07 $\mu\text{g/g}$ in Accipitridae and 19.61 $\mu\text{g/g}$ in Falconidae, which could be toxic;

however, in Strigidae it was 5.19 µg/g. Mean concentrations of Ni and Cu in our study were many fold higher than those found in the feathers of birds of prey in Belgium (Dauwe et al., 2003). Concentrations of heavy metals in birds above the threshold level may result in behavioral changes, reproductive dysfunction, and increased chances of disease (Scheuhammer, 1987). Reproductive dysfunction could be a direct result of exposure, causing

smaller clutch size, hatching failure, mortality of nestlings, and egg-shell thinning, and overall influenced the health of birds (Nyholm, 1993; Belskij et al., 1995; Jassens et al., 2003; Dauwe et al., 2004). Metal concentrations detected during our study from various raptors and regions of Pakistan might cause population declines of these species; proper management and control of these pollutants in the environment are therefore required.

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