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Avian feathers as a non-destructive bio-monitoring tool of trace metals signatures: A case study from severely contaminated areas

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HIGHLIGHTS

- Trace metals were assessed in avian feathers collected from industrial areas.
- Concentrations among the highest ever found in similar samples were recorded.
- Such high concentrations of Cr, Pb, Cd may affect avian reproduction.
- Avian feathers are a convenient sampling tool for assessing metal contamination.

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ABSTRACT

The concentrations of trace metals were assessed using feathers of cattle egrets (*Bubulcus ibis*), collected within two industrial areas of Pakistan, Lahore and Sialkot. We found, in order of descending concentration: Zinc (Zn), Iron (Fe), Nickel (Ni), Copper (Cu), Cadmium (Cd), and Manganese (Mn), Chromium (Cr), Arsenic (As), and Lithium (Li), without any significant difference (except Fe, Zn, and Ni) between the two areas. The concentrations of trace metals, we recorded were among the highest ever reported in the feathers of avian species worldwide. The concentrations of Cr, Pb, Cd were above the threshold that affects bird reproductive success. The high contamination by heavy metals in the two areas is due to anthropogenic activities as well to natural ones (for As and Fe). The bioaccumulation ratios in eggs and feathers of the cattle egret, their prey, and the sediments from their foraging habitats, confirmed that avian feathers are a convenient and non-destructive sampling tool for the metal contamination. The results of this study will contribute to the environmental management of the Lahore and Sialkot industrial areas.

1. Introduction

In some developing countries, environmental degradation is increasing day by day as a consequence of the exponential growth of the human population, and of technological developments (Qadir and Malik, 2011; Xu et al., 2013; Ayyamperumal et al., 2006). Environmental contamination, by heavy metals among other chemicals, involves great health risks for all living organisms, humans and wildlife (Salzano and Angelone, 2013; Ullah et al., 2014; Klaassen, 2013). Heavy metals are non-biodegradable environmental contaminants that may accumulate in the upper levels of the food chains, in relation to the habitat and dietary preferences of each species (Boncompagni et al., 2003). Trace metals such as Chromium (Cr), Copper (Cu), Mercury (Hg), Arsenic (As), Cobalt (Co), Manganese (Mn), Selenium (Se), Zinc (Zn) and many others enter the food chain, and have the capacity to biomagnify

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(Burger and Gochfeld, 2004; Bostan et al., 2007). Exposure to these heavy metals is widely described to threaten the reproductive success of many avian species in the wild (Malik and Zeb, 2009).

The need to check for the effects of heavy metals on the environment has led to various bio-monitoring strategies, involving the use of indicators, i.e. particular organisms that may reflect the contamination of their ecosystem (Kalisinska et al., 2003; Burger and Gochfeld, 2007). Birds have been recognized since the 1960s as the potential bioindicators of environmental pollution (Erwin and Custer, 2000). Their potential is primarily due to the position of some bird species at the top of the food chain, so that any change in the lower trophic level is signaled by their response (Boncompagni et al., 2003). Many bird species live in close association with human activities, are exposed to environmental contaminants, and may suffer from the resulting toxic effects (Malik and Zeb, 2009). The concentrations of heavy metals in feathers, droppings and eggs of birds have been documented worldwide over the years (Dauwe et al., 2005; Malik and Zeb, 2009; Hashmi et al., 2013), and metal contamination was shown to affect bird populations (Gragnaniello et al., 2001; Muralidharan et al., 2004). Bird feathers offer several advantages as bioindicators of metal exposure and feather collection is non-invasive. The concentration of heavy metal is higher in feathers than in the other tissues, and hence easier to detect and quantify, because birds excrete considerable amounts of metals through feather moult (Malik and Zeb, 2009; Zamani-Ahmadmahmoodi et al., 2010). Moreover, feathers from newly born chicks indicate local contamination, derived mostly from food collected locally by their parents during the short period of egg formation and chick development (Boncompagni et al., 2003; Muralidharan et al., 2004). Waterbirds that breed in colonies provide additional advantages as bioindicators of pollution: easy sampling (Burger and Gochfeld, 2000a,b); a limited foraging range around their colony site, thus allowing inference about the source of contaminants (Burger et al., 2004); and dependence on specific habitat and prey resources (Fasola et al., 1998).

In recent years, it has become evident that environmental contamination is severe in various areas of Pakistan (Malik and Zeb, 2009), where industrial, urban and agricultural activities are major sources of pollution (Movalli, 2000; Qadir et al., 2008). For some agricultural areas, moderate concentrations of metal contamination have recently been reported using the feather of the cattle egret (*Bubulcus ibis*) as bioindicator (Malik and Zeb, 2009; Ullah et al., 2014). For the industrial areas of Pakistan, however, information on metal exposure is scarce, while, these areas might be severely contaminated by the vast quantities of urban and industrial waste from tanneries, pharmaceuticals, surgical and steel manufacturing factories. Moreover, these areas are also reported to serve as dumping sites for persistent organic pollutants (POPs) and expired chemicals after the ban imposed by Stockholm (2001).

In this paper, we assess the contamination by trace elements of prime environmental concern (As, Cd, Cr, Pb, Mn, Li, Fe and Zn) in two of the main industrial areas of Pakistan, near the cities of Sialkot and Lahore, that are presumably exposed to severe pollution, and for which we had already assessed the persistent organic pollution (Khan et al., 2013). We used as bio-indicator the cattle egret, a widespread, colonial, and predatory waterbird (Ciconiiformes: Ardeidae). In order to monitor the contaminant concentrations and their levels throughout the food chain, we collected diverse samples, cattle egret's eggs, chick feathers, its prey, and the sediments of its foraging areas in the vicinity of the breeding sites. Our results offer baseline data for South Asia, where contamination studies are still scanty; emphasize potential threats to avian populations; and provide information to the authorities concerned with the mitigation of environmental contamination, which might ultimately threaten human health in the severely polluted industrial areas of Pakistan.

2. Materials and methods

2.1. Study area

Samples were collected at two study areas, Lahore and Sialkot (Fig. 1), two major cities of Punjab Province, and, two of the major industrial hubs of Pakistan (Aftab et al., 2000). These study areas are described in detail by Khan et al. (2013). In the last decades, the exponential population growth of this region, coupled with the development of extensive industrial activities, tanneries, steel factories, leather garments and sport equipment manufacture, has resulted in widespread environmental degradation (Qadir et al., 2008). Sialkot city was reported to generate 19 million $m^3\ year^{-1}$ of waste water in 2007 (World Wildlife Fund), and Lahore city generated 1350 million $L\ d^{-1}$ of wastewater (Mahmood and Malik, 2014). No water treatment facility exist in these two areas, and the wastewater, drained into natural streams, ponds, open field and croplands, can be expected to produce severe environmental contamination.

2.2. Sampling plan and analytical procedures

In the Lahore area, cattle egret samples were collected near Dad Pura village, 1 km from Kalashah Kaku interchange (31°57′02″N, 74°24′14″E). This area hosts significant industrial activities, including still mills, chipboard and rubber manufactures, leather tanning and pigment factories. In the Sialkot area, another cattle egret colony (32°29′50″N and 74°32′10″E) was sampled near Khajori Wala village, 3.5 km from Sialkot city. In both study area(s), rice is the main crop, and it receives water from canals that might get polluted by the waste coming from nearby industrial areas. The egrets forage in the rice fields and in other water bodies, and are therefore exposed to the toxic metals through food intake and dust ingestion.

The samples, sediments, prey and eggs, were collected at the two study areas, using the methods detailed by Khan et al. (2013). Moreover, samples of 8–12 breast feathers were collected at two cattle egret's colonies from one chick per nest, when the chick was 10–15 d old and the external attachment of contaminants should still have been low. The feather samples were packed in polythene bags and stored in refrigerator (at −20 °C) till further analysis.

Before analysis, feathers were washed with deionized water, followed by acetone, in order to remove loosely adherent exogenous contamination. The washed feathers were put in metal-free polyethylene vials and dried in oven for 24 h at 60 °C. Dry weight was determined using a digital balance. Each sample was cut into small pieces with a stainless steel knife, and digested as described by Dauwe et al. (2002) with few modifications. All containers were soaked in 10% (v/v) HNO_3 overnight and rinsed with ultra-pure water by three times. Each sample was carefully weighed, then digested with 1 mL GR grade 65% (v/v) HNO_3 (Merck, Germany) overnight, and at the next day with 1 mL GR grade 30% (v/v) H_2O_2 (Merck, Germany). The samples, mixed and sealed in Teflon microwave digestion tubes, were digested in an accelerated microwave digestion system (Mars CEM, CEM Corporation, Matthews NC, USA) at 800 W, 120 °C for 10 min and then 800 W, 170 °C for 30 min. Trace metals were measured using a flame atomic absorption spectrophotometer (Perkin Elmer-AA240FS Fast Sequential Atomic Absorption Spectrometer). Table S-1 represents the instrumental conditions for the measurements of the analytes by FAAS. The other, samples, sediments, prey and eggs, were digested and analysed by the same method.

2.3. Quality assurance and quality control

A quality control (QC) sample was prepared by mixing aliquots of each sample, that were therefore broadly representative of the

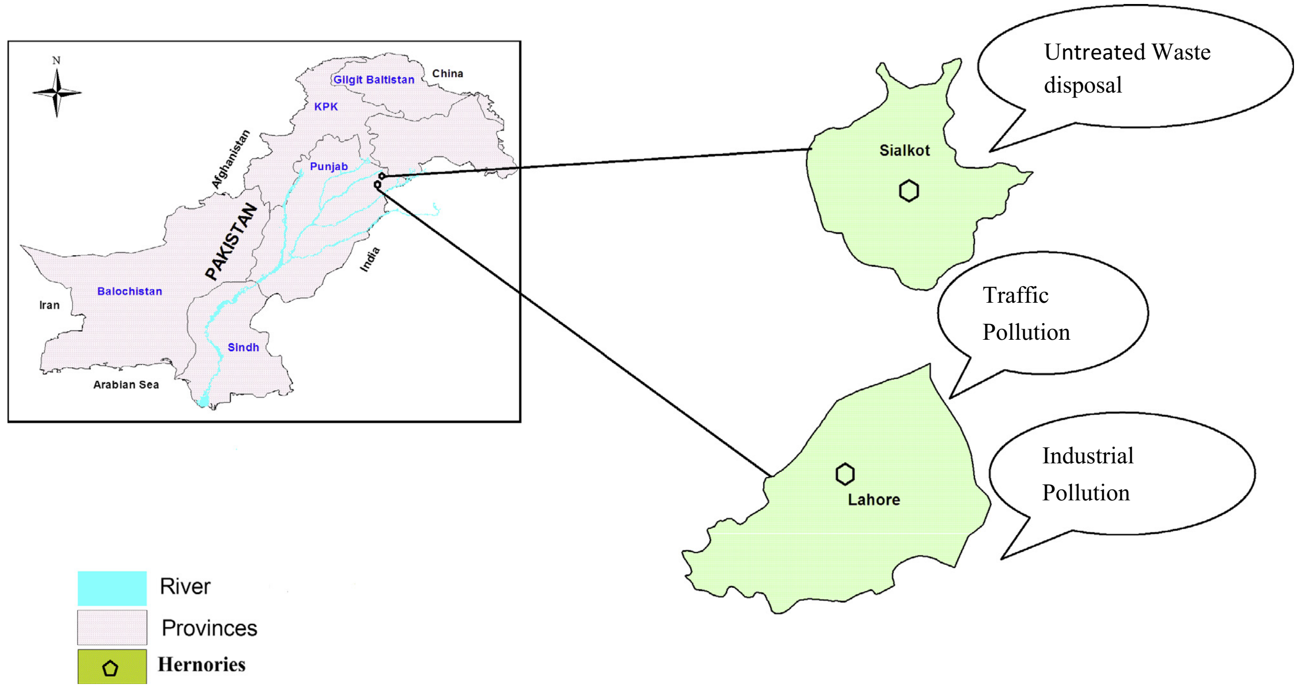

Fig. 1. Study areas near the cities of Sialkot and Lahore, Pakistan, and main sources of pollution.

whole samples set. The QC sample was injected every 15 samples, in order to assess the instrumental stability. The variations of metal concentrations of the QCs were <15%. Reagent blanks were also included in each batch of analyses, in order to check for any contamination of the different samples extracts. Average values of three replicates were taken for each determination. The precision of the analytical procedures was expressed as relative standard deviation (RSD) which ranged from 5% to 10% and was calculated from the standard deviation divided by the mean. Calibration curves were prepared separately for each metal, using different concentrations (i.e. 0.5 ppm, 1 ppm, 2 ppm, 5 ppm and 10 ppm) of standard solutions. The working solutions were daily prepared by appropriate dilutions of the standard stock solution, using a mixture of 65% (v/v) HNO_3, 30% (v/v) H_2O_2 and H_2O (v/v/v = 1:1:3). The calibration curves, relative coefficients (r^2) of calibration curves, the limit of detections (LOD), and the instrumental conditions, are detailed in Table S-1. The instrument was set to zero concentration for all samples, using a reagent blank. Each determination was based on the average values of three replicate measurements. Chemicals, stock solutions and reagents were obtained from Merck and were of analytical grade. All glassware before use were washed with distilled water, soaked in nitric acid (30%) overnight, rinsed in deionized water (Behropur B25), and air-dried.

2.4. Statistical analyses

Descriptive statistical analysis was conducted using Statistica 5.5 (Statsoft Inc., 1999) All the differences were tested at the 0.05 significance level, if not stated otherwise. Descriptive statistics were calculated from imputed values. One-way ANOVA, followed by Tukey's HSD post hoc test on http://www.vassarstats.net/, was applied to multiple comparisons among different sites, using Minitab 15. Spatial distribution maps of important metals, that had been found in all previous studies, were produced using Geostatistical Analyst-extension/tool of ArcGIS$^{(R)}$ (Version 10.1).

3. Results and discussion

Our results on concentrations and bioaccumulation of trace elements are detailed in Tables 1 and 2, and in Figs. 2 and 3. The chemical profile of trace metals was as follows: Zn > Fe > Pb > Ni > Cu > Cd > Mn > Cr > As > Li. The concentration of trace metals in the feathers did not differ significantly in our two study areas, Sialkot and Lahore, except for Zn, Ni, and Fe ($p > 0.05$; Fig. 2). In the following sections, we present our result for each metal. Furthermore, we compare all the data on metal contamination that are available for Pakistan (Fig. 3), and we discuss our results in relation to the metal concentrations reported in various avian species from different parts of the world (Table 3).

3.1. Concentration profiles and risk to the avian fauna

3.1.1. Copper

The concentrations ($\mu g\ g^{-1}$) of Cu in feathers at two studied areas were ranged from 28–74 and 42–93, for both Lahore and Sialkot, respectively. However, the measured Cu values in feather samples at Lahore study area were a bit lower than from Sialkot. In general, our results indicate higher environmental concentrations from the industrial areas, in agreement with studies conducted elsewhere (Dauwe et al., 2002; Janssens et al., 2002; Eens et al., 1999). Conversely, recorded Cu concentration from our study areas was higher than those reported for other birds and for the cattle egret (Dmowski, 1999; Hashmi et al., 2013; Migliorini et al., 2004; Muralidharan et al., 2004; Ullah et al., 2014). Cu is an essential element, required in low concentration to meet various physiological needs, but high dose and chronic exposure may pose serious deleterious effects including reproductive, respiratory, gastrointestinal, hematological, hepatic, endocrine, and ocular damage as well as causing cancer (ATSDR, 2004; Chen and Wei, 1993). The permissible limit of Cu in various environmental matrices are: 1.3 mg L^{-1} in drinking water (USEPA, 2010), 0.1 mg m^{-3} in fumes, 1 mg m^{-3} in mist and dust (Occupational Safety and Health Administration, 2002; Klaassen, 2013). These guideline values

Table 1
Concentration (geometric means in μg g^{-1} dry weight ± SE) of trace metals in sediment, prey, eggs, and feathers from two heronries of cattle egrets, Lahore and Sialkot, Pakistan. No data from Sialkot were available for prey.

	Lahore				Sialkot		
Trace Metals	Sediment	Prey	Eggs	Feathers	Sediment	Eggs	Feathers
As	11.3 ± 0.9	5.2 ± 1.2	2.3 ± 1.2	21.4 ± 4.2	10.6 ± 5.3	2.1±.39	19 ± 2.5
Cd	6.3 ± 0.4	3.3 ± 1.4	1.7 ± 1.6	41 ± 1.3	7.5 ± 0.3	1.2±.45	37.6 ± 1.2
Cr	18.2 ± 4.5	2.4 ± 1.2	2.4 ± 2.1	21.1 ± 18.2	14.7 ± 4.5	3.9 ± 1.6	19 ± 13.4
Cu	19.6 ± 2.9	20 ± 13.5	4 ± 1.9	52.8 ± 16.1	18.4 ± 6.5	3.9 ± 1.6	62.7 ± 13.6
Fe	1348 ± 376	32.2 ± 16.5	38.5 ± 22	156 ± 154	1463 ± 477	40 ± 29.4	283 ± 305
Li	13 ± 2.6	6.8 ± 4.7	0.11 ± 0.1	7.3 ± 0.2	12.7 ± 3.4	0.57 ± 0.3	7.45 ± 0.4
Mn	158 ± 39	49 ± 11.8	4.6 ± 1.3	16 ± 14.3	141 ± 56.3	5.4 ± 1.5	21.9 ± 13.2
Ni	36.1 ± 5.1	13 ± 2.8	4.8 ± 1.2	41.6 ± 7.2	36.5 ± 4.9	5.2 ± 2.6	84.8 ± 4.2
Pb	115 ± 6.1	37.8 ± 5.7	47 ± 12.5	297 ± 11	124 ± 18.7	41 ± 8.4	286 ± 18.4
Zn	123 ± 55	163 ± 23.3	48 ± 12	529 ± 95	97.2 ± 39	56.2 ± 9.8	226 ± 75

Table 2
Bioaccumulation ratios, as proportion of the concentration in eggs, in feathers, and in prey, in relation to the concentration in the sediments. No data from Sialkot were available for prey.

	Feathers (n = 21)		Eggs (18)		Prey (n = 10)
Trace Metals	Lahore (n = 11)	Sialkot (n = 10)	Lahore (n = 7)	Sialkot (n = 11)	Lahore (n = 10)
As	1.89	2.02	0.21	0.20	0.46
Cd	6.49	5.00	0.28	0.17	0.54
Cr	1.16	1.29	0.14	0.27	0.13
Cu	2.70	3.40	0.21	0.21	1.02
Fe	0.12	0.19	0.03	0.03	0.01
Li	0.56	0.59	0.01	0.05	2.48
Mn	0.10	0.16	0.03	0.04	0.31
Ni	1.15	2.32	0.13	0.14	0.36
Pb	2.57	2.30	0.41	0.33	0.33
Zn	4.29	2.33	0.39	0.58	1.32

show that our avian population is at severe reproductive and physiological health risk due to high Cu exposure (Carpenter et al., 2004).

3.1.2. Manganese

Mn is involved in several biochemical reactions in living organism, and acts as an essential micronutrient. It originates from igneous rocks, but urban emissions including traffic and waste incineration contribute towards the Mn burdens in the environment (Zayed et al., 1999). In our study, Mn ranged <BDL-35 (mean; 21) μg g^{-1} at Sialkot, and <BDL-30 (mean; 16) μg g^{-1} at Lahore. The Mn contamination was more frequent in samples from Sialkot with ~90% detection frequency, versus 70% in samples from Lahore. The concentrations found in our study were comparable to those in the feather of species of the family Ardeidae from Pakistan and China (Malik and Zeb, 2009; Burger, 1993) but were higher than those reported from agricultural areas of Pakistan (Boncompagni et al., 2003; Ullah et al., 2014). These high concentrations of Mn can be attributed to exposure of birds via contaminated dust and to ingestion with food. Diesel fuel burning might be a source of Mn pollution in our study areas, as it is used as an anti knocking agent in leaded gasoline (Qadir et al., 2008). Untreated dumping of traffic waste and other industrial effluents in the foraging area might be another possible source of Mn. Toxicity caused by Mn can produce anemia, micromelia, limb twisting, hemorrhage, stunted growth, and behavioral disorders (ATSDR, 2012; Summers et al., 2011; Gibbs et al., 1999). Its effect on the cattle egrets is unknown and needs further investigation (Malik and Zeb, 2009).

3.1.3. Nickel

Ni was detected among all the samples. Its concentrations ranged between 30–47.5 and 77–89 μg g^{-1}, respectively in Lahore and Sialkot, and differed significantly between the two areas. These concentrations of Ni were comparable to those reported by Nighat et al. (2013) in predatory avian feathers for a nearby area, but were higher than those for other, agricultural areas of Pakistan (Boncompagni et al., 2003; Malik and Zeb, 2009; Ullah et al., 2014). These results suggest that industrial activities including Ni–Cr platting, ghee production, electrical appliance manufacturing, tanning, and battery production in the vicinity of catchment are the main source of Ni via both surface run off and river flow (Hanif et al., 2005). In our study area, both of the sampling sites were located in industrial zones; with manufacturing of electrical appliances such as Ni–Cd batteries and the dumping of untreated

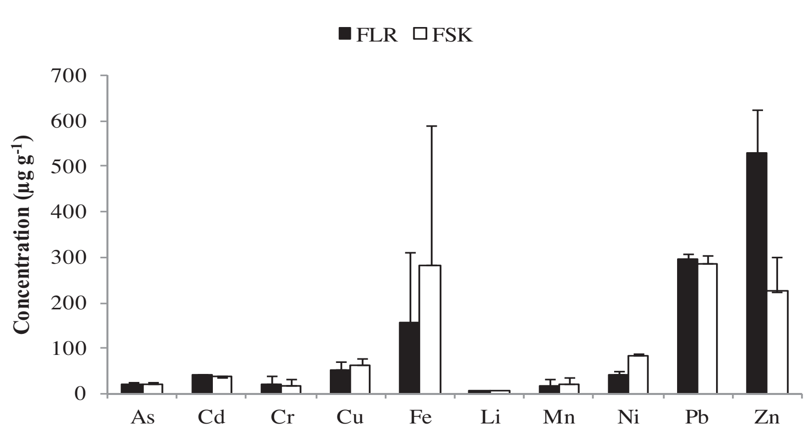

Fig. 2. Comparison of heavy metals in feathers of cattle egrets from both studied site locations (Lahore; FLR and Sialkot; FSK).

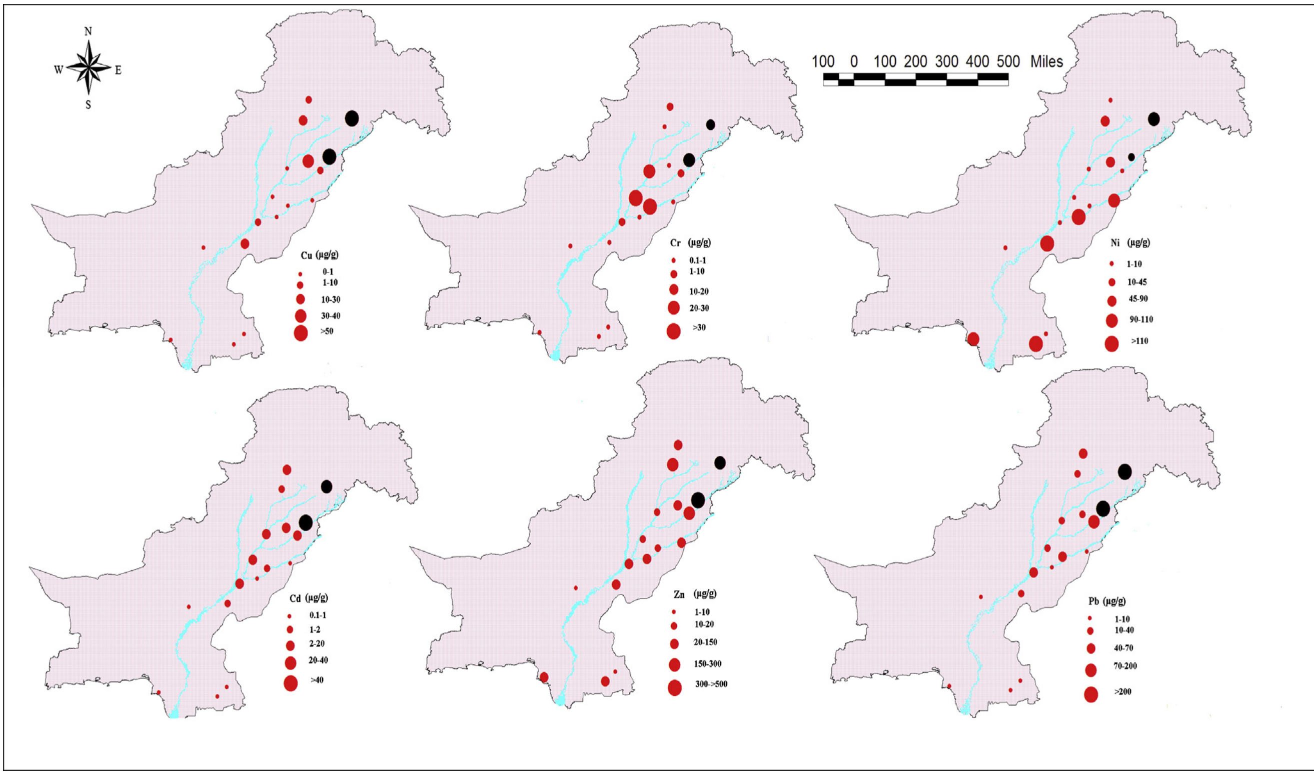

Fig. 3. Concentration of trace metals (μg g^{-1}) in the avian fauna feathers from this study (black dots) and from previous ones, 2000–2013 (red dots). Detailed values are in Table 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Concentration of trace metals (μg g^{-1}) in avian feathers from this and from previous studies (2000–2013).

Author	Study area	Cu	Cr	Mn	Fe	Ni	Cd	Zn	Li	Pb As	As
Worldwide											
Burger and Gochfeld (2007)	Amchitka		0.97	1.24			0.07			1.14	
Kim and Koo (2008)	Yeongjong Island, Korea	10.62		5.26			0.8	83.58		13.88	
Deng et al. (2007)	Badachu Park, China	5.45	1.85	7.1		2.5	0.1	276		5.4	
Kim and Koo (2007)	Korea	11.65		11			172	32.5		0.5	
Burger et al. (2008)	Prince William Sound		1.6	1.29			0.05			1.21	
Zhang et al. (2006)	Poyang China		0.1					139.23			
Dauwe et al. (2004)	Belgium	3				0.3	0.2	54.6		2.6	
Dauwe et al. (2002)	Belgium	78.5					9.8	242		252	
Burger and Gochfeld (2000)	Northern Pacific Ocean		1.8	1.02			127.65			1.24	
Dauwe et al. (2000)	Belgium	5.3					0.1	123		2.1	
Pakistan											
Nighat et al. (2013)	Southren	16.9				153.4	1.8	122.2		19.5	24
	Central	21.5				90.3	3.4	127.2		21	8.2
	Northern	16.8				42.4	1.1	158.5		18.6	12
Ullah et al. (2014)	Trimun Headwork	0.9	26.2	2.1	200.6	0.1	3.3	18.5	1.03	30	
	Shorkot	0.2	30.8	3.1	126.8	0.2	3	18.8	1.5	32.5	
	Mailsi	0.2	35.8	2.4	99.8	0.2	1.7	10.7	0.86	43.1	
Malik and Zeb (2009)	Chenab River	4	6.6	26.9	181.8	9	3.1	133.8	0.9	37.5	
	Ravi River	3.7	7.1	15.3	106.3	8.1	2.4	155.2	0.7	76.5	
	Rawal Lake	4	5.4	16.9	117.7	7.8	2.7	138.2	0.8	60.2	
Boncompagni et al. (2003)	Pakistan		0.7	2.45				113.84			
Movalli (2000)	Bahawalnagar					104	0.1	105		1	
	Bahawalpur					113	0.1	113		3	
	Chachro						0.1			1	
	Jackabad						0.1			1.5	
	Karachi					104		104		0	
Present study	Lahore	52.8	21.1	16	156.3	41.7	41.1	529.9	7.3	284	25
	Sialkot	59.8	19.1	21.8	338.5	83.8	37.6	219.9	7.522	296	20

toxic waste might have resulted into elevated concentrations of Ni in the environment. Excess Ni is reported to affect feather moulting (Malik and Zeb, 2009). The Ni concentrations found in our study are much higher than those reported by Deng et al. (2007) and Dauwe et al. (2004). Ni exposure can cause allergic reactions, stomach ache, chronic bronchitis, and carcinogenesis in humans (ASTDR, 2005).

3.1.4. Chromium

Chromium is no longer considered an essential element for animals and may cause deleterious effects on reproductive health of different avian species (Malik and Zeb, 2009). Cr was frequently detected in our samples. Its concentrations ($\mu g\ g^{-1}$) ranged from BDL to 41 (mean; 21) Lahore, and from BDL to 44 (mean; 19) in Sialkot, and did not differ between the two study sites. Worldwide, there are few reports on the incidence of chromium in the feather of wild avian species. However, comparison of our dataset with other documented studies clearly indicated a severe Cr contamination in our study areas. Similar concentrations in avian feathers were reported by Ullah et al. (2014) from Pakistan and by Burger and Gochfeld (1995) from China. Our study found high concentrations of Cr in comparison to those reported for Asia by Movalli (2000); Deng et al. (2007); Zhang et al. (2006). High concentrations of Cr, albeit lower than in our study, were also detected in feathers of little egrets (*Egretta garzetta*) from Haleji Lake and from Taunsa, Pakistan (Boncompagni et al., 2003). Our study area is famous for leather industry, and the high concentrations of Cr can be related to the extensive use in tanning process of Cr salts, disposed in the environment as effluent and sludge (Qadir et al., 2008), and ultimately taken up by biota through the food chain (Khan et al., 2013). The uptake of Cd, Cr and Pb in bird feathers may come from secretions of the uropygial gland, of the nasal gland, and may be enhanced by the keratin binding affinity of the heavy metals (Morais et al., 2012). The avian populations in Lahore and Sialkot districts are at risk, since they show concentrations higher than the threshold (2.8 $\mu g\ g^{-1}$) reported by Burger and Gochfeld (2000).

3.1.5. Cadmium

Cadmium is also a non-essential element, present in the environment as a result of anthropogenic activities, with deleterious effects for birds on egg formation, testicular damage, oviduct malfunctioning and kidney damage (Malik and Zeb, 2009; Birge et al., 2000; Burger, 2008; Eisler, 1985). Higher concentrations of Cd may also affect the metabolic processes through replacement of essential elements at the active sites of biologically important molecules, thus indirectly inducing nutritious deficiencies (Furness, 1996). We found high concentrations ($\mu g\ g^{-1}$) of Cd, ranging 30–43 (mean; 41) at Lahore, and 30–35 (mean; 37) at Sialkot. The high levels of these contaminants in our study may be related to the extensive industrial activities in the two areas. Cd originates from erosion of surface deposits of minerals containing this element (Malik et al., 2010), and from anthropogenic sources including the purification of ores from smelters and mines, as well as from commercial products such as batteries, paints, coatings on metal devices, and plastic stabilizers (Qadir et al., 2008). These findings are consistent with other studies that reported high accumulation in urban soils of Cd, as well as Pb and Zn, (Malik et al., 2010) and in organisms (Qadir et al., 2008) from similar regions. However, the Cd concentrations in our samples were far higher than those reported by other studies from agricultural and sub-urban areas from Pakistan (Ullah et al., 2014; Nighat et al., 2013; Malik and Zeb, 2009; Movalli, 2000). On the other hand, similar concentrations in avian feathers were reported for Belgium (Dauwe et al., 2002), for northern Pacific Ocean (Burger and Gochfeld, 2000), and even higher concentrations from Korea (Kim and Koo, 2007). In our study, about 90% of the samples exceeded the Cd concentrations that are considered a threshold limit (2 $\mu g\ g^{-1}$) for potential threat to avian populations (Burger and Gochfeld, 2000).

3.1.6. Lead

Pb is a non essential element that is deposited mainly into calcareous tissues and in feathers (Smichowski et al., 2006; Markowski et al., 2013). Pb has been widely reported as indicator of metal pollution caused by anthropogenic sources (Metcheva et al., 2006), including sewage disposal, fuel combustion, waste incineration, paints, vehicles, polishing, leather industry and oil spills (Jerez et al., 2011; Markowski et al., 2013). In our study, the avian feathers exhibited the highest concentrations among all the studied metals, with concentrations ($\mu g\ g^{-1}$) from 270–307 (mean 297) for Lahore, and 290–315 (mean 286) for Sialkot. The concentrations in our samples are in accordance with a study for Belgium (Dauwe et al., 2002), which also showed very high Pb-concentrations in the feather of blue tit (270 $\mu g\ g^{-1}$) and great tit (250 $\mu g\ g^{-1}$). The Pb concentrations reported in other studies from Pakistan (Movalli, 2000; Boncompagni et al., 2003; Malik and Zeb, 2009; Ullah et al., 2014; Nighat et al., 2013) were far lower than those measured in our case. The reason for such high levels in our study may be related to extensive industrial activities and urban-traffic, since the study areas serves as dumping grounds for untreated waste material. A recent study for a similar region (Qadir and Malik, 2011), reported severe contamination by Pb from point sources including effluents from tanneries, pharmaceutical industries, municipal sewage, where large quantities of Cr and Pb salts are released into the environment, causing the deterioration of the aquatic biotopes. Cattle egret fed on earthworms and insects collected from the rice field surrounding their colonies, suggesting that either the earthworms are living in lead-rich soils and/or are exposed to air-borne lead contamination from smelters (Bostan et al., 2007). Our results indicates that Pb concentrations in feathers (250 $\mu g\ g^{-1}$), were well above the threshold (4 $\mu g\ g^{-1}$) that causes adverse effects in birds, reproductive problems and stunned growth (Burger and Gochfeld, 2000a).

3.1.7. Arsenic

Arseniosis due to As toxicity is a major health disaster and has become a major concern in South Asian countries (Bhattacharya et al., 2007; Chakraborti et al., 2004; Kapaj et al., 2006). Our study area is characterized as one of the most As-contaminated areas of the world, with the highest As-concentrations ever reported in the drinking water (>100 $\mu g\ L^{-1}$), and with widespread cases of Arseniosis in the human population (Farooqi et al., 2007). Inorganic As causes acute toxicity, sub-chronical toxicity, developmental toxicity, reproductive toxicity, genetic toxicity (Chakraborti et al., 2004), immunotoxicity (Sakurai et al., 2004), cellular toxicity, biochemical toxicity and chronic toxicity (Mudhoo et al., 2011; Schwarzenegger et al., 2004). The main route of exposure to inorganic As is drinking water (Mudhoo et al., 2011; National Research Council, 2001), and chronic ingestion, which may cause cancer and is associated with skin, lungs, kidney and bladder malfunction (ATSDR, 2003; USEPA, 2010). In our study, avian feather had As concentrations from 17–25.5 $\mu g\ g^{-1}$ (mean; 21.4) at Lahore, and 10–24.5 (mean; 19) at Sialkot. The values from our study are agreement with those in avian feathers (Nighat et al., 2013) and in fish (Waheed et al., 2012) of similar regions. As exposure in our study area may be contributed by several industrial processes, including preservative, electronics, mining, and pesticide and herbicide manufacturing (Waheed et al., 2012), through drinking water contamination. There are only few reports on As exposure for the avian fauna, nevertheless, such information is of prime importance to reflect the risk of this priority environmental pollutant.

3.1.8. Lithium

Li occurs naturally in several minerals including pegmatite, brines, and clays, but it is not freely available to organisms. Man-made sources include several industries, ceramics, glass, alloys, alkaline storage batteries, preparation of Li compounds, welding, brazing, and enameling (Ullah et al., 2014). Previous studies for Pakistan (Ullah et al., 2014; Malik and Zeb, 2009) reported considerable concentrations of this toxic metal in the feathers of waterbirds including the cattle egret, but even higher were the concentrations we recorded at Lahore (~7.5 $\mu g\,g^{-1}$) and Sialkot (7 $\mu g\,g^{-1}$). The main source of Li pollution in these industrial areas may be related to the discharge of Li in effluents from glass, pottery, enameling, and alloy industries (Hashmi et al., 2013). Moreover, the rapid urbanization of our study areas involved welding and brazing activities, which ultimately may have resulted in unintentional exposure to Li fumes that may cause irritation and pulmonary edema (Malik and Zeb, 2009).

3.1.9. Zinc

Zn concentrations in feathers ranged 400–650 $\mu g\,g^{-1}$ (mean 529) at Lahore, and 94–315 (mean 226) at Sialkot. Zn, an essential element that plays important roles in different metabolic reactions, originates both from natural and from anthropogenic sources (Ullah et al., 2014; Morais et al., 2012; Strezov, 2012). Malik et al. (2010) suggested that Zn in our study areas come from salts used in tanning industries in Sialkot, and from uncontrolled waste disposal from heavy mechanical industry from Lahore. The Zn concentrations reported by our study are in agreement with those found in avian feathers by Boncompagni et al. (2003) and Nighat et al. (2013) for Pakistan, by Dauwe et al. (2002) for Belgium, and by Zhang et al. (2006) and Deng et al. (2007) for China. However, the concentrations found in our study are far higher than those previously reported for other areas of Pakistan (Movalli, 2000; Malik and Zeb, 2009; Ullah et al., 2014). No effects of high concentrations of Zn in biological systems are known.

3.1.10. Iron

Fe is an essential element for animals. It originates naturally from rock and soil, but man-made sources such as smelting, iron ores, vehicles, and machinery are also contributing towards the Fe-levels into the environment, although inorganic Fe is not bioavailable. Fe sources includes the use of iron containing materials in construction, automobiles, containers and steel mills (Hashmi et al., 2013; Ullah et al., 2014). In our study, Fe concentrations in feathers ranged from 15–430 $\mu g\,g^{-1}$ (mean 156) at Lahore and 85–870 (mean 283) at Sialkot. These concentrations are comparable with those previously reported for different areas of Pakistan (Malik and Zeb, 2009; Ullah et al., 2014).

3.2. Spatial distribution of toxic metals using bird feathers

The concentrations of each trace metal in bird feathers throughout Pakistan (Fig. 3 and Table 3) helps to assess the extent of contamination in the different regions, and to evaluate the suitability of the avian bioindicators as surveillance tools of metal exposure. Our spatial analysis compared sites that included different types of land use (i.e., industrial, urban, and agricultural), because our aim was to assess the overall impact of industrial activities on the environment. The agricultural and sub urban premises receive industrial waste through a network of rivers, canals, and nallah (stream) therefore we believe that the maps in Fig. 3 reflect the metal burden throughout the local environment. Our approach is supported by the results by Malik and Zeb (2009), who reported very high levels of toxic metal from the agricultural areas of the Punjab that were located near the River Chenab and River Ravi. The same authors related such high contamination to river flows from Lahore, Sialkot, Faisalabad and other cities into the catchment area. Nighat et al. (2013) reported relatively lower levels from Chakwal and few other cities which are not recipient of waste from any water body, but very higher levels in Lahore and Faisalabad. Therefore, we believe that the maps in Fig. 3 indicate the impact of industrialization and urban activities for each region.

The nation-wide comparison (Fig. 3) clearly indicates that our study areas near the industrial regions of Lahore and Sialkot are heavily polluted by the trace metals. Our results are in agreement with the severe contamination reported by Nighat et al. (2013) for central Punjab (Lahore, Sialkot and Gujrat, Qasoor, Gujranwala), and by Qadir and Malik (2011) for the Nallah Aik and Nallah Palkhu, which receive waste from Lahore and Sialkot, two districts that are highly populated and produce huge amounts of openly disposed waste. On the other hand, Malik and Zeb (2009) and Ullah et al. (2014) documented moderate pollution levels for sub-urban and agricultural areas of Punjab, and Movalli (2000), and Boncompagni et al. (2003) reported for southern Pakistan, the concentrations of trace metals that were comparable to the minimum values found in our study.

3.3. Bioaccumulation of toxic metals

In order to check the usefulness of bird feathers as non-destructive tool for the surveillance of trace metal contamination, we calculated the bio-accumulation ratios (Table 2), using the approach by Boncompagni et al. (2003). Our results indicated that the ratios in the feathers were significantly ($p < 0.05$) higher than those of the related eggs and prey samples (for all the metals except Li and Mn, which showed higher values for prey in few cases). Among the trace metals, the bioaccumulation pattern in feathers in descending order was: Cd, Zn, Cu, Pb, Cr, Ni, As, Li, Fe, and Mn. Cd showed bioaccumulation ratios of 5–6.5 followed by Zn (2.3–4.29), Cu (2.7–3.4) and Pb (~2.5), Cr (~1.5); while Li (0.56–0.59), Mn (0.1–0.16), and Fe (0.12–0.19) exhibited lower values. Avian feathers are confirmed as effective samples for measuring heavy metal contamination in the environment (Malik and Zeb, 2009).

The uptake and accumulation of the toxic metals by birds may depend on multiple factors including diet, age, and metabolism (Boncompagni et al., 2003). Birds deposit Pb, Cd and Cr into their growing feathers, thus limiting adverse effects, since these toxins bonds to the feather keratin and become physiologically isolated (Malik and Zeb, 2009). Moreover, deposition of these metals in their calcareous tissues is a major pathway for Pb removal in female birds (Lam et al., 2005; Scheuhammer, 1987). Cu and Zn are preferentially deposited in the feathers only if the concentrations of these metals are above high concentrations. In summary, feathers are an effective pathway to excrete toxic metals as Pb, Cd, Cr, Zn, and Cu (Honda et al., 1986).

Our study supports the proposal by Burger (1993), Boncompagni et al. (2003), and Malik and Zeb (2009), that toxic metals are subject to bioaccumulation at both higher and lower concentrations. Although some elements are essential to avian physiology (Eeva and Lehikoinen, 2002; Larison et al., 2000). Heavy metal pollution may damage their reproduction (Janssens et al., 2002).

4. Conclusions

Our study assessed the concentration of trace elements in one of the most contaminated areas of Pakistan, for which scarce information existed, using cattle egrets as biomonitor. The results provide evidence that feathers are efficient and non-destructive samples for the study of biomagnifications of trace metals throughout the food chain, with higher concentrations found in bird feathers than in their eggs, diet, and in sediments. Between the two study sites, Sialkot and Lahore, the trace metal concentrations did not differ significantly, and this suggests a similar exposure at both sites to toxic chemicals released from nearby industrial and urban areas. Our analysis on toxic metals contamination in different parts of Pakistan, may help to assess the status of the Country towards Global toxic metals emissions.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.06.068.

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