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Heavy metal contamination in feathers of house crow (*Corvus splendens*)

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Abstract
The present study was planned with an objective to detect the level of heavy metals in feathers of House Crow from agrifield areas of district Ludhiana and Sangrur. In feathers of House Crow, 8 heavy metals were detected through ICAP-AES, out of which As, Cd, Pb were heavy metals and Cr, Cu, Ni, Zn, Mn were essential elements. The concentration of all metals varied significantly at .05% level of significance among the different districts. The significant variations in metal level at different locations throw light on the fact that there are significant fluctuations in the level of contamination of the environment where these birds live and feathers can be used as an indicator of such contamination in wild birds without harming them.

Keywords: Agrifields, contamination, heavy metal, house crow, indicator

Introduction
Heavy metals have always been a part of our environment, usually present in very low concentrations and are persistently discharged into the environment from regular sources like volcanic action or weathering of rocks. Heavy metals can be sub-grouped into Manganese (Mn), Iron (Fe), Cobalt (Co), Zinc (Zn) and Molybdenum (Mo)- small concentrations of these elements are necessary for development and life cycles of living beings, yet are harmful if present in high doses. Lead (Pb), mercury (Hg) and cadmium (Cd) are found to be lethal even if present in small concentrations [31].

Ecological contamination by heavy metals, for example, zinc (Zn), copper (Cu), iron (Fe), nickel (Ni) and lead (Pb) has gotten significant consideration [25]. Heavy metal contamination from anthropogenic activities represents a possible risk to the human wellbeing and natural life through bioaccumulation in the food chain [34]. Through natural processes, heavy metals discover their path to the soil, as a result contaminate water assets and flora [1]. These heavy metals that pile up in the water and flora afterwards through food chain accumulate in final consumers, including birds [14].

The occurrence of heavy metals (arsenic, cadmium, nickel, zinc) in biological system can have pernicious impacts since they don't debase and have long half-lives [3]. These heavy metals, to a huge degree, are scattered in nature through industrial effluents, organic fertilizers, waste, blazing, vehicles and power genesis [2]. The manufacturing of colours, medications, agrochemicals, polythenes and batteries; electroplating and release of untreated effluents from various factories additionally cause contamination [25].

Once these metals through food chain enter the body, they might be eliminated or accumulated. Birds can deposit metals either in their plumes (feathers) [5] or can sequester in their eggs, which jeopardize the developing embryo [8]. Feathers are helpful indicators of metal pollution because they are easy to collect non-invasively and we can store them indefinitely. Moreover, the extent of body burden that is in feathers is generally steady for each metal, a moderately high extent of the body burden of some metals is put away in the feathers [5], because of their affinity for the sulfhydryl rich keratin protein and melanin pigments and there is typically a high correlation between levels of contaminants in the food of birds and levels in their feathers [28]. The levels of metals in eggs are derived from females and represent exposure in waters close to the breeding grounds and also mobilization of stored metals from past intake [7]. Various reviews have been done concentrating on the bioaccumulation of heavy metals in the tissues and plumes of birds [5, 21, 18, 29]. Naccari [29] studied the aggregation of both toxic and non-toxic heavy metals in Common buzzard (*Buteo buteo)* and revealed that *B. buteo* can be

utilized as a reasonable bioindicator for observing the contamination caused by these heavy metals. The levels of heavy metals in synanthropic living beings have been likewise evaluated in numerous nations: Spain [39], Korea [30], France [33] and Malaysia [20]. Because of the persistence of lead in the soils of regions with high anthropogenic exercises and its exchange through the food chains, Janaydeh [20] commented that toxic heavy metals may still stay as an environmental concern for House Crow.

Intake of high levels of heavy metals like Pb, Ni, Zn, Cd and Cu can be lethal to bird species and impact the endocrine system, respiratory system, stomach, kidneys, breeding, behavioural response, shedding, migration, catalysts required in haemoglobin formation and development rates. Nickel is utilized in metal plating, alloy making, as a catalyst in chemical reactors and batteries. It is deposited in rivers and streams through rundown and is taken up by animals and birds, influencing their respiratory system, birth imperfections and harm to DNA. Zinc is utilized in alloying, colouring, assembling electric items, insect sprays, makeup and could result in decreased species diversity, abundance and evenness. Cu is an essential trace element that is metabolically controlled, but its abundance result in extra distressing ecological conditions [31]. Heavy metals have been very much examined in the aquatic food chain [37] and result in poor rearing as well as high mortality for falcons and ospreys. The terrestrial food chains are relatively less studied [32].

Keeping in view the lack of information on majority of Indian bird species on the effects of environmental contaminants, present study was carried out to provide information on the presence of such contaminants in the eggs and feathers of House Crow.

Materials and Methods

Heavy Metal Analysis

The fallen feathers of House Crow were also collected and stored in the plastic bags. The samples were labelled appropriately with the source, time and date of collection and then stored in refrigerator for further use.

Preparation of digestate of egg and feather samples for analysis

0.5g of feather sample were taken and 10ml of diacid (3:1 ratio of conc. Nitric acid: Perchloric acid) was added. A blank was also prepared without the samples. These samples were then digested using Hot Plate at 100^0 C for 30 minutes. The final volume was made to 25ml with distilled water and the solution was filtered.

Elemental Analysis

The digested samples were analyzed for heavy metals (arsenic, cadmium, chromium, copper, nickel, manganese, lead and zinc) by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICAP-AES) at Department of Soils, PAU, Ludhiana.

Calculations

The readings taken on ICAP-AES were converted into parts per million (ppm) as per the following formula:

$$\frac{(\text{Reading of sample} - \text{reading of blank}) \times \text{Total volume used}}{\text{Weight of sample}}$$

Results and Discussion

In this study, the concentrations of 8 heavy metals (arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) were analyzed in feathers of House Crow by using by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICAP-AES) at Department of Soils, PAU, Ludhiana. All these heavy metals were considered for this analysis because they are widely reported to be potentially toxic to birds.

Heavy metal levels were analyzed in 0.5 g samples of feathers of House Crow which included pooled samples of about 7-10 feathers of House Crow and in 1g samples of eggs. For this purpose, total 16 feather samples were analyzed, of which 8 feather samples were collected from agricultural fields of selected villages of Sangrur district and 8 were collected from agricultural fields of PAU, Ludhiana. Out of 8 heavy metals, As, Cd and Pb are considered as non-essential and toxic elements, whenever present. While Cr, Cu, Mn, Ni, Zn are considered as essential elements as they are needed in body to perform some specific functions. These essential elements are also toxic when present above threshold concentrations in birds. All these 8 heavy metals have been found to be present in feathers of House Crow. The concentrations of each heavy metal in feathers are displayed in Table 1. The presence of these heavy metals in feathers of House Crow indicated that House Crow was exposed to the heavy metals contamination in the habitat. For heavy metals in all House Crow feathers analyzed, mean metal concentrations varied in the order: Zn>Mn>Pb>Cu>Ni>Cr>Cd>As. Thus in all samples, maximum concentration was of Zn and minimum of As which reveals the possible bioaccumulation of these heavy metals affecting House Crow in a chronic manner by increasing reproductive dysfunction or causing endocrine disruption.

The heavy metal analysis in feathers collected from Ludhiana showed that the mean concentration of Zn was maximum (75.043±28.245 ppm), followed by Mn (17.921±7.610 ppm), while Cd mean concentration was minimum (0.215±0.078 ppm) metal. Pb, Cu, Ni, Cr and As showed moderate concentrations (Table 1). The heavy metal analysis in feathers collected from selected villages of Sangrur district showed that the mean concentration of Zn was highest of all the metals analyzed (416.337±18.815 ppm), followed by Mn (110.55±8.851 ppm), while As showed the lowest mean concentration (0.215±0.078). Pb, Cu, Ni, Cr and Cd depicted moderate mean concentrations (Table 1). The comparative non-parametric statistical analysis conducted between heavy metal concentrations in the analyzed feathers of PAU, Ludhiana and selected villages of Sangrur district in Table 2. The non-parametric Mann-Whitney U test confirmed that the mean concentrations of all 8 heavy metals in feathers differ significantly from each other by $P<0.05$ at Ludhiana and Sangrur (Table 2).

Arsenic is a non-essential element found in water, soil, air, plants and all living tissues. A large amount of Arsenic is released into environment anthropogenically, mainly through agricultural practices including the application of pesticides. Range of as concentration varied from 0.1 to 0.725 ppm and 0.85 to 2.1 ppm in feathers collected from PAU, Ludhiana and selected villages of Sangrur district. The concentrations of heavy metals are usually low (1 ppm wet weight, which approximately represents 3 ppm dry weight) in most living organisms [4]. In the present study the concentration of As was below this range in the feathers of House Crow at different locations of Punjab. Arsenic mean concentrations in the present study were higher than those in Common eider feathers (0.161 ppm) from the Aleutian Islands, USA [7] and from Amchitka and Kiska, USA (0.163 ppm) [7] in feathers

collected from selected villages of Sangrur, but lower in feathers collected from Ludhiana. Signs of acute arsenite poisoning in birds include asthenia, ataxia, jerkiness, slowness, falling, hyporeactivity, ptosis, huddled position, fluffed feathers, loss of righting reflex, immobility and tetanic seizures [17]. Highly toxic inorganic As found in some seabirds may also act as an endocrine disruptor, can cause the death of an individual, exert sub lethal effects, or may result in reproductive failure [23].

Table 1: Range and Mean concentration of heavy metals in feathers of House Crow collected from agricultural fields of PAU, Ludhiana and selected villages of Sangrur district (dry weight in ppm).

Location Heavy → metals↓	Feathers collected from agricultural fields of PAU, Ludhiana		Feathers collected from agricultural fields of selected of Sangrur district		Toxic Range (ppm)
	Range (ppm)	Mean±S.E (ppm)	Range (ppm)	Mean±S.E (ppm)	
Arsenic	0.1-0.725	0.293±0.088	0.85-2.1	1.287±0.142	>3 (Braune and Noble 2009)
Cadmium	0.05-0.55	0.215±0.078	1.05-1.55	1.337±0.071	>0.01 to 0.02 (Burger 1993)
Chromium	1.1-4.9	1.921±0.507	7.45-14.15	9.543±0.711	>2.8 (Burger and Gochfield 2000))
Copper	1.025-18.825	6.815±2.385	16.25-24.4	19.468±0.983	>100 (Jayakumar and Muralidharan 2011)
Manganese	2.025-54.35	17.921±7.610	82.05-155.5	110.55±8.851	-
Nickel	0.625-4.2	1.775±0.540	8.45-13.8	10.275±0.679	>1 (Braune and Noble 2009)
Lead	11.025-20.225	13.946±1.332	35.35-52.6	44.406±2.448	>4 (Burger and Gochfield 2000)
Zinc	8.85-199.725	75.043±28.245	368.85-505.1	416.337±18.815	>1.20 (Gasaway and Buss 1972)

Table 2: Non-parametric procedure (Mann-Whitney test) showing difference in concentration of heavy metals in feathers of House Crow collected from agricultural fields of PAU, Ludhiana and selected villages of Sangrur district.

	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
Mann-Whitney U	.000	.000	.000	5.000	.000	.000	.000	.000
Wilcoxon W	36.000	36.000	36.000	41.000	36.000	36.000	36.000	36.000
Z	-3.366	-3.378	-3.376	-2.836	-3.361	-3.363	-3.361	-3.361
Asymp. Sig. (2-tailed)	0.001	0.001	0.001	0.005	0.001	0.001	0.001	0.001
Mann-Whitney Test								
Grouping Variable: Districts								

Cd is one of the most abundant nonessential metals present in the environment because of its use in industrial activities. If absorbed into the organism through the digestive and pulmonary systems, cadmium forms complexes with proteins, in which it is easily transported and stored, mainly in the liver and kidneys and in smaller quantities, in the pancreas, intestines and bones. In current study, Cd mean concentrations in feathers collected from selected villages of Sangrur were higher than those in feathers of Little egret (0.635 ppm) and Black crowned Night heron (0.553) from Northern Italy [10] and Common Bulbul (0.48) from Nigeria [15]. Range of Cd concentration varied from 0.05 to 0.55 ppm and 1.05 to 1.55 ppm in feathers collected from PAU, Ludhiana and selected villages of Sangrur. Burger (1993) suggested that feather levels of Cd that are associated with adverse effects would range from 0.01 ppm (shearwaters) to 0.02 ppm (terns). In the present study the concentration of Cd was above this range in the feathers (collected from PAU, Ludhiana and selected villages of Sangrur district) of House Crow suggesting this metal would cause adverse affects to the House Crow, which induce kidney toxicity, lesions in intestinal tissue, disruption of calcium metabolism, decreased food intake, and thinning of eggshells [19]. Cd is listed in the "most dangerous trace element category". The high values of Cd in the feathers of selected villages of Sangrur district indicate that the environment is highly stressed with respect to Cd. Cd is not an essential element for animals and may induce deficiencies of essential elements through competition at active sites in biologically important molecules. At higher concentrations, it may cause kidney damage, altered behaviour, suppression of egg production, egg shell thinning and testicular damage [12]. At the population level, reduced growth rates of bones and fledging success were correlated with exposure to elevated Cd concentrations in feathers [36].

Lead in feathers can enter by two routes. Endogenously through food; it reflects the available lead in blood at the time of feather formation [5]. Avian species are particularly at risk because they are exposed to Pb through ingestion of grit, soil intake from preening or ingestion of contaminated food near ranges. Exogenously, deposited from the environment on the feather between formation and sampling or molt, therefore an indicator of environmental contamination for sedentary species like House Crow [8]. Sources of Pb in the environment include fuel additives, lead pigments in paints, batteries, pipes and glazed ceramic food containers. Inorganic Pb may be absorbed through the skin, the respiratory system and gastrointestinal tract. Range of Pb concentration varied from 11.025 to 20.225 ppm and 35.35 to 52.6 ppm in feathers collected from PAU, Ludhiana and selected villages of Sangrur. According to Burger and Gochfield [6] Pb levels of 4 mg/kg in bird feathers are associated with negative effects such as delayed parental and sibling's recognition, locomotion, impaired thermoregulation, depth perception, lowered nestling survival and abnormal feeding behaviour. Such high Pb concentration in feathers provides evidence for the significant pollution of habitats with this heavy metal and for its probable bioaccumulation in feathers of House Crow, primarily from the habitat in which this bird lives and in eggs from food. Range of Pb concentrations from the present study was higher than Pb concentrations observed by Mansouri [27] in *Egretta gularis* feathers (2.17 to 16.45 ppm) and by Gushit [15] recorded in 16 different bird feathers (0.45 to 2.81 ppm). Range of Pb concentrations was also higher than Pb concentrations observed by Burger [7] in Common eider eggs (0.002 to 3.800 ppm). Pb is known as calcium- formations seeking element, readily accumulates in bones, hairs, feathers and nails and is not metabolically regulated which makes it potentially important for monitoring of anthropogenic pollution. Pb may interact with calcium metabolism in birds. Pb causes behavioural defects in animals due to its toxic effects on the nervous system, and may result in decreases in survival, growth rates, poorer fledging success, learning and

metabolism [9].

Pb and Cd have no documented positive role in most living organisms, it is rather associated with breeding failure, decreased body weight and reproduction impairment in some egret and heron species [5, 16].

Cr, Cu, Mn, Ni, Zn are essential elements, they are necessary for the metabolism but can however cause adverse effects when their concentrations in the organism increase. The essential element are required by each tissue in order to perform number of functions, as physiologically they are required for the gradual increase of the movement of nestling, they are necessary for feather formation and growth, even for ossification which appeared with bone growth [17]. Usually, essential heavy metals are non-toxic; however, various studies have showed the toxicity of the essential heavy metals in wild birds at certain concentrations [35, 11].

Cr is an essential mineral that plays a role with insulin to regulate blood sugar levels. Cr is found naturally in rocks, plants, soil and volcanic dust, humans and animals. Cr is generally produced by industrial processes. Range of Cr concentration varied from 1.1 to 4.9 ppm and 7.45 to 14.15 ppm in feathers collected from PAU, Ludhiana and selected villages of Sangrur. In the present study the concentration of Cr was above the range 3 ppm in feathers given by [4] of House Crow at different locations of Punjab. Cr concentration of 2.8 ppm in feathers of birds might be associated with adverse effects [6]. Thus this showed that Cr lies in high concentrations in feathers of House Crow. Cr also produces adverse effects on embryonic development, hatching and viability of the birds [23]. In present study mean concentration of Cr was higher than Common eider feathers (1.790 ppm) observed by [7].

Cu is an essential element involved in the formation of several key enzymes for the release of energy inside the cell and contributes to the function of many antioxidants. Cu is widely used in industries and agriculture. Due to this, Cu concentrations in the environment have increased. Mean concentration of Cu in feathers collected from selected villages of Sangrur district is found to be higher than that observed by Janaydeh [20] in House Crow (15.57 ± 5.47 ppm), by Naccari [29] in Common Buzzard (9.24 ± 1.83 ppm) and by Komosa [24] in Gray Plover (19.1 ± 5.01). Conversely, Manjula [26] observed greater mean concentrations of Cu in the feather samples of Cattle Egrets (61.10 ± 5.88) and Greylag goose (35.70 ± 41.00), as compared to the results of the current study. Jayakumar and Muralidharan [2] found that Cu concentrations of 100 ppm in liver tissues of mute swan did not exhibit any sign of Cu toxicity. The positive sign of this is that the concentration of Cu detected in all samples tested in study shows no sign of toxicity because it is below the 100 ppm adverse effect thresh hold limit for Cu in birds.

Manganese supports the immune system, regulates blood sugar levels and is involved in the production of energy and cell reproduction. Burning of diesel fuel is one of the sources of Mn in environment. Teratogenic effects (such as micromelia, twisted limbs, and hemorrhage and neck defects), behaviour impairments, altered growth rates and reduction of hemoglobin formation have been linked to sub lethal Mn exposure in animals and avian embryos. According to Burger [7] Mn mean concentration in feathers of Common eider was (10.500 ppm). In contrast to this, Mn mean concentration recorded in present study was much higher in feathers (17.921 ppm in Ludhiana and 110.55 ppm in Sangrur) of House Crow as compared to concentration reported in feathers of Common eider.

Nickel plays important role in proper functioning of liver in animals. Ni is one of the trace elements widely distributed in the environment, being released from both natural and anthropogenic activities. Range of Ni concentration varied from 0.625 to 4.2 ppm and 8.45 to 13.8 ppm in feathers collected from PAU, Ludhiana and selected villages of Sangrur district. In the present study the concentration of Ni was above the range 3 ppm in feathers and 1ppm in eggs given by Braune and Noble [4] of House Crow at different locations of Punjab. Although Ni had the moderate mean concentration among the 8 heavy metals analyzed, it was high enough to harm House Crow and thus depicting the high exposure House Crow to Ni in environment. According to Van Wyk [40], Ni can affect the respiratory system of birds, causing asthma, as well as birth defects, vomiting and damage to DNA. Mean Ni concentration in feathers of House Crow (12.15 ppm) from Pakistan by Janaydeh [20] was within range of Ni concentration recorded in feathers of House Crow collected from selected villages of Sangrur district.

Zn is an important component of the enzymes responsible for metabolism of proteins and carbohydrates. It is released to the environment from both natural and anthropogenic sources. The primary anthropogenic sources of Zn in the environment are related to mining and metallurgic activities and use of products containing Zn, like cloth clips or staples, zippers, keys, nails, plumbing nuts and some antirust paints, shampoos and skin preparations. Range of Zn concentration varied from 8.85 to 199.725 ppm and 368.85 to 505.1 ppm in feathers collected from PAU, Ludhiana and selected villages of Sangrur district. According to Taggart [38] threshold limits for Zn do not exist. However, Gasaway and Buss [13] reported that the threshold level of Zn in birds is 1.20 ppm. Therefore, in the present study, the Zn concentrations were significantly higher than the threshold limits in all feather and egg samples of House Crow. Greater concentration of Zn in selected villages of Sangrur district depicted high level of exposure of House Crow to Zn in the environment. But, in villages of Sangrur, source of Zn is unknown as no mining or metallurgic activities occur there. Mean concentrations of Zn in present study were found to be higher than those reported by Naccari [29] in feathers of Common Buzzard (60.01 ppm), by Tejedor [39] in feathers of Gray Plover (131.9 ppm) and Janaydeh [20] in House Crow feathers (206.31). Zinc is an extremely toxic heavy metal and has potential to harm birds. Although, birds require certain concentration of Zn to remain healthy, too much zinc is very toxic and can cause disfunctioning of the liver, pancreas, kidney and even result in death.

Conclusion

The toxic heavy metals were found to be present in all feather samples of House Crow collected from different sites. The concentration of heavy metals and other essential metals varied in feather samples from site to site. Estimates of all 8 heavy metal concentrations in feathers of House Crow collected from villages of Sangrur were much higher than the levels of feathers collected from Ludhiana. This reveals that House Crow may be exposed to heavy metal contaminations in Sangrur environment and as a result considered as one of the reason for decreasing numbers of House Crow in Sangrur.

The significant variations in heavy metal concentration among different locations throw light on the fact that there are significant fluctuations in the level of contamination of the environment where the House Crow live and feathers can be used as an indicator of such contamination in House Crow without harming them. Thus, House Crows is an important

model species in ecological research and a suitable and cost-effective bioindicator for environmental contamination.

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