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RESEARCH ARTICLE

Heavy metal contamination in excreta of avian species from Ludhiana district of Punjab

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Abstract

The present study was planned with an objective to detect the level of heavy metals in excreta of five bird species; House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret collected from agrifield areas of district Ludhiana. In excreta of all the birds 16 elements were detected through ICAP-AES, out of which As, B, Cd, Pb were heavy metals and Fe, Cr, Cu, Ni, Zn, Mn, Mg, Ca, P, S, K, Na were essential elements. The concentration of all metals except K, Mg and Na varied significantly at 5% level of significance among the species. In all the five species, the range of arsenic (0.422-2.142 ppm) and boron (24.50-43.20 ppm) was observed above normal range i.e. 0.01-0.2 and 0.13-5 ppm respectively. The level of cadmium was excreted above normal range (0.02-1.5 ppm) by Cattle egret (1.880 ppm) only. The concentration of lead ranged from 3.9 to 11.73 ppm in five species. Species specific differences in metal level may be attributed to the trophic level relationships. The significant variations in metal level among the species throw light on the fact that there are significant fluctuations in the level of contamination of the environment where these birds live and excreta can be used as an indicator of such contamination in wild birds without harming them.

Introduction

Bird population is particularly susceptible to the effects of anthropogenic activities on the environment. Over the years among wildlife, birds have served as bioindicator for a number of environmental contaminants particularly heavy metals (Mochizuki et al., 2002) as they are visible, widely distributed in the ecosystem, sensitive to toxins and high on the food chain. Birds can rid the body of heavy metals through the faeces or by depositing them in the uropygial gland, salt gland (Burger and Gochfeld, 1985) and feathers (Burger, 1993). Females can also eliminate heavy metals by sequestering them in their eggs, which may jeopardize the developing embryo. The faecal material as an indicator of metal pollution has been shown to reflect the metal pollution level well in environment and food items, indicating especially the food chain contamination (Dauwe et al., 2004).

In India, studies on heavy metal contamination in birds are limited. Moreover, the capturing and killing of birds is legally banned by Govt. of India according to Wildlife Protection Act 1972, therefore any analytical studies on the tissues and eggs of these organisms are beyond the reach of scientists working in this area. Thus, an excreta of bird is an alternative source, which if analyzed can give an assessment of the harmful impact of environmental contaminants on these organisms. Therefore, the present studies were planned in order to assess the level of heavy metals in the excreta of birds with different feeding habits and also to determine if excreta can be used as non-invasive method to monitor metal level in environment.

Materials and methods

The present study was planned as per the mandate of All India Network Project on Agricultural Ornithology funded by Indian Council of Agricultural Research (ICAR), New Delhi.

Collection of excreta of birds

Dry excreta of five bird species namely House Crow, Corvus splendens; Common Myna, Acridotheres tristis; Blue Rock Pigeon, Columba livia; Ring Dove, Streptopelia decaocto and Cattle Egret, Bubulcus ibis were collected from their roosting, foraging and nesting sites in agrifield areas of Punjab Agricultural University and dairy farm of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana respectively during March, 2013 to September, 2013. The collected excreta samples were labeled appropriately with the source, time and date of collection.

Digestion of excreta samples

The dry excreta samples of different bird species were weighed and 0.4 g excreta sample of each species in three replicates were taken. To each sample 4 ml of conc. HNO_3 was added. A blank was also prepared without the excreta sample. These samples were then digested in Perkin's microwave at 121°C for 52 minutes. The final volume was made to 25 ml with distilled water and the solution was filtered.

Elemental analysis of digested excreta samples

The digested samples were analyzed for the elemental composition especially, for the presence of various heavy metals by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICAP-AES) in Department of Soil Sciences, PAU, Ludhiana. Each analysis was done in triplicate while single blank was run for 5 samples. The readings taken on ICAP-AES were converted into parts per million (ppm= µg/g). The reported values were compared with the recommended normal range values of metals for avian species by Wisconsin Veterinary Diagnostic Laboratory (WVDL), Toxicology centre, United States.

The data collected was represented as mean and standard error and was subjected to one way analysis of variance (ANOVA) followed by Tukey HSD ($p<0.05$) to assess whether heavy metals vary significantly among the different avian species.

Results

Concentration of metals in excreta

The elemental composition of faecal pellets of different bird species (House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret) has shown the presence of 16 elements in total i,e. Arsenic (As), Boron (B), Calcium (Ca), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Nickel (Ni), Phosphorus (P), Lead (Pb), Sulphur (S) and Zinc (Zn) in present studies. Out of these As, Cd, B and Pb are included in the category of heavy metals which are non-essential elements and toxic whenever present. The others like Fe, Cr, Cu, Ni, Zn, Mn, Mg, Ca, P, S, K and Na are essential elements needed in body to perform some metabolic activities. But all these essential elements are also toxic when present above threshold levels in human beings and birds too. The concentration of all metals except K, Mg and Na varied significantly at 5% level of significance among the species (Table 1).

Arsenic

The level of As was found to be 0.725 ± 0.008, 0.783 ± 0.140, 0.422 ± 0.062, 0.567 ± 0.178 and 2.142 ± 0.05 ppm in the pellets of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret respectively. The normal range for As is 0.01-0.2 ppm. In present studies, the concentration of As was above the normal range in all species and was recorded minimum in granivorous birds and maximum in insectivorous bird.

Boron

The level of B in the faecal matter of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret was observed to be 43.20 ± 4.5, 32.59 ± 4.085, 30.6 ± 4.062, 24.50 ± 1.940 and 35.19 ± 0.35 ppm respectively. The normal recommended range of B for avian species is 0.13-5 ppm. The concentration of B in all the samples was higher than the normal range with a maximum value in excreta of House Crow, an omnivorous bird.

Calcium

In the faecal matter of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret, the level of Ca was detected to be 13127 ± 775.54, 17696 ± 858.37, 14610 ± 356.37, 9025.33 ± 3377.1 and 5577.33 ± 161.32 ppm respectively. In present studies the highest level of Ca was reported in the excreta of Common Myna and lowest in the excreta of Cattle Egret.

Cadmium

Cd concentration was 0.120 ± 0.01, 0.553 ± 0.168, 0.192 ± 0.026, 0.564 ± 0.262 and 1.880 ± 0.066 ppm in the faecal matter of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret respectively. The level of Cd was highest as well as above the normal range (0.02-1.5 ppm) in Cattle Egret among the five spp.

Chromium

In the faecal matter of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret, the level of Cr was detected to be 2.65 ± 1.123, 12.85 ± 1.072, 6.747 ± 1.373, 9.401 ± 1.133 and 16.29 ± 0.133 ppm respectively. Cr concentration was lowest in House Crow and highest in Cattle Egret.

Copper

The level of Cu was found to be 9.86 ± 1.655, 26.19 ± 1.596, 119.47 ± 52.60, 20.74 ± 3.629 and 22.74 ± 0.734 ppm in the pellets of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret respectively. In Blue Rock Pigeon the concentration of Cu was very high (5-10 fold) as compared to other species.

Iron

In the faecal matter of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret, the level of Fe was detected to be 1076.87 ± 565.82, 6202.67 ± 1150.0, 4855.67 ± 1166.7, 5648 ± 1127.1 and 8345.67 ± 226.35 ppm respectively.

Potassium

In the faecal matter of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret, the level of K was detected to be 13545 ± 3612.6, 7815 ± 1236.3, 13253.33 ± 724.68, 6647 ± 1835.0 and 9158.67 ± 223.55 ppm respectively. The K level was highest in House Crow and lowest in Ring Dove.

Magnesium

The level of Mg was found to be 3103 ± 918.45, 4512.67 ± 771.85, 5599.33 ± 213.75, 3679 ± 255.45 and 3434.33 ± 78.26 ppm in the pellets of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret respectively. Magnesium concentration was highest in Blue Rock Pigeon and lowest in House Crow.

Manganese

In the faecal matter of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret, the level of Mn was detected to be 45.58 ± 21.78, 164.37 ± 9.927, 147.33 ± 13.58, 194.67 ± 0.9171 and 212.3 ± 6.44 ppm respectively. The maximum level of Mn was observed in Cattle Egret and minimum in House crow.

Sodium

The level of Na in the faecal matter of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret was observed to be 3187.33 ± 1221.7, 2569.33 ± 461.09, 1607.33 ± 28.95, 823 ± 125.83 and 2390 ± 139.84 ppm respectively. The recommended normal range of Na in avian species is 2800-3750 ppm. In all samples the concentration of sodium was reported in normal range.

Nickel

In the faecal matter of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret, the level of Ni was detected to be 2.564 ± 1.369, 8.530 ± 0.323, 6.43 ± 1.318, 6.67 ± 0.455 and 12.51 ± 0.412 ppm respectively. Cattle Egret was observed to excrete maximum level of nickel.

Phosphorus

The level of P was found to be 4690 ± 2535.0, 8112 ± 645.86, 13378.67 ± 1083.7, 10831.33 ± 397.38 and 7164.33 ± 103.22 ppm in the pellets of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret respectively.

Lead

In the faecal matter of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret, the level of Pb was detected to be 3.90 ± 1.305, 11.73 ± 2.102, 5.753 ± 0.676, 5.775 ± 0.638 and 8.325 ± 0.099 ppm respectively. The maximum level of lead was observed in Common Myna followed by Cattle Egret in present studies. The concentration of Pb ranging from 3.9 to 11.73 ppm in present studies indicates high exposure of this metal to the birds of five species.

Sulphur

The level of S was found to be 2472.33 ± 752.83, 3685.67 ± 250.96, 4320.33 ± 208.07, 2804 ± 270.37 and 3821.33 ± 35.22 ppm in the pellets of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret respectively. However no toxic range has been defined for S in avian species. The maximum concentration of this metal was seen in excreta of Blue Rock Pigeon.

Zinc

The level of Zn was found to be 52.63 ± 17.614, 189.23 ± 13.842, 154.17 ± 12.18, 155.67 ± 13.670 and 137.53 ± 3.38 ppm in the pellets of House Crow, Common Myna, Blue Rock Pigeon, Ring Dove and Cattle Egret respectively. The normal levels of Zn in birds vary between 100 and 200 µg/g (Van wyk et al., 2001). In present studies the concentration of Zn metal was in normal range in all the samples.

S. No.	Element	House Crow	Common Myna	Blue Rock Pigeon	Ring Dove	Cattle Egret

1	Arsenic	0.725 ± 0.008[a]	0.783 ± 0.140[a]	0.422 ± 0.062[a]	0.567 ± 0.178[a]	2.142 ± 0.05[a]
2	Boron	43.20 ± 4.5[a]	32.59 ± 4.085[a]	30.6 ± 4.062[a]	24.50 ± 1.940[a]	35.19 ± 0.35[a]
3	Calcium	13127 ± 775.54[a]	17696 ± 858.37[a]	14610 ± 356.37[a]	9025.33 ± 3377.1[a]	5577.33 ± 161.32[a]
4	Cadmium	0.120 ± 0.01[a]	0.553 ± 0.168[a]	0.192 ± 0.026[a]	0.564 ± 0.262[a]	1.880 ± 0.066[a]
5	Chromium	2.65 ± 1.123[a]	12.85 ± 1.072[a]	6.747 ± 1.373[a]	9.401 ± 1.133[a]	16.29 ± 0.133[a]
6	Copper	9.86 ± 1.655[a]	26.19 ± 1.596[a]	119.47 ± 52.60[a]	20.74 ± 3.629[a]	22.74 ± 0.734[a]
7	Iron	1076.87 ± 565.82[a]	6202.67 ± 1150.0[a]	4855.67 ± 1166.7[a]	5648 ± 1127.1[a]	8345.67 ± 226.35[a]
8	Potassium	13545 ± 3612.6	7815 ± 1236.3	13253.33 ± 724.68	6647 ± 1835.0	9158.67 ± 223.55
9	Magnesium	3103 ± 918.45	4512.67 ± 771.85	5599.33 ± 213.75	3679 ± 255.45	3434.33 ± 78.26
10	Manganese	45.58 ± 21.78[a]	164.37 ± 9.927[a]	147.33 ± 13.58[a]	194.67 ± 0.9171[a]	212.3 ± 6.44[a]
11	Sodium	3187.33 ± 1221.7	2569.33 ± 461.09	1607.33 ± 28.95	823 ± 125.83	2390 ± 139.84
12	Nickel	2.564 ± 1.369[a]	8.530 ± 0.323[a]	6.43 ± 1.318[a]	6.67 ± 0.455[a]	12.51 ± 0.412[a]
13	Phosphorus	4690 ± 2535.0[a]	8112 ± 645.86[a]	13378.67 ± 1083.7[a]	10831.33 ± 397.38[a]	7164.33 ± 103.22[a]
14	Lead	3.90 ± 1.305[a]	11.73 ± 2.102[a]	5.753 ± 0.676[a]	5.775 ± 0.638[a]	8.325 ± 0.099[a]
15	Sulphur	2472.33 ± 752.83[a]	3685.67 ± 250.96[a]	4320.33 ± 208.07[a]	2804 ± 270.37[a]	3821.33 ± 35.22[a]
16	Zinc	52.63 ± 17.614[a]	189.23 ± 13.842[a]	154.17 ± 12.18[a]	155.67 ± 13.670[a]	137.53 ± 3.38[a]

Table 1: Level of different metals including heavy metals (ppm) in excreta of different bird species from agrifield areas of PAU, Ludhiana

[a]Significant difference among the species, P≤0.05
The data is represented as mean ± standard error of three samples of each species.

Discussion

Toxicological consequences of heavy metals

In present studies, the toxic heavy metals have been found to be present in excreta of all species. Some heavy metal ions that are known to be potentially toxic include arsenic, cadmium and lead and also essential metals such as iron, manganese, copper, zinc, selenium, nickel and cobalt (Duruibe et al., 2007). The concentrations of heavy metals are usually low (1 ppm wet weight, which approximately represents 3 ppm dry weight) in most living organisms (Braune and Noble, 2009). The toxic elements can be harmful to birds even at low concentrations when ingested over a long period of time. The type of food eaten by birds plays a predominant role in the amount of As they would accumulate (Lebedeva, 1997). Arsenic is poisonous to multicellular life and has recently also been accused of being a potent endocrine disruptor, with sub-lethal effects leading to disruption of reproduction or death of some sea birds (Kunito et al., 2008). Boron compounds have moderate acute toxicity and the symptoms include depression, ataxia, congestion, convulsions and weight loss (Weir and Fisher, 1972). Stanley et al. (1996) also found that 900 mg/kg dietary boron in Mallard Ducks caused reduced hatching success by more than 42%, reduced duckling weight and growth with 47% reduction in number of ducklings produced per female. However, calcium is essential for living organisms and contributes to skeletal and egg shell formation in birds (Roudybush and Grau, 1991) but if consumed in excess then it can prove fatal. Cadmium is listed in the "most dangerous trace element category" and is present in both the environment and food with long persistence and high toxicity. Animals excrete more than 99% of the Cd they ingest mainly in the faeces (Lee et al., 1994). Cd concentration measured in the present study was much lower than those found in faecal samples of Vulture (13.93 ± 1.18 ppm) from Venezuala (Bravo et al., 2005). In present studies, mean Cd concentration varied from 0.120–1.88 ppm in the faecal pellets of different spp. and were within the range reported by Hashmi et al. (2013) in the eggshells of Cattle Egret. Mora and Anderson (1995) regarded chromium concentrations of 6.5-17 µg/g to be below the threshold for biological effects. The concentration of Cr in all the samples was below threshold level in present studies. However, in larger amounts and in different forms, chromium can be toxic and carcinogenic. Cr toxicity in mallard produces adverse effects in embryo development, viability and hatching (Kerte'sz and Fa'ncsi, 2003). Bravo et al. (2005) reported the Cu level to be 20.26 ± 0.41 and 14.50 ± 8.60 ppm in the faeces and plasma of Black Vulture (Coragypus atratus) respectively without any toxicity. Acute Cu poisoning occured in liver of Canada Goose (Branta canadensis) at Cu concentrations of 187 to 323 µg/g (Henderson and Winterfield, 1975). Therefore, in present studies the concentration of Cu was below the toxic range in all the species. Fe has been reported to play an important role in all the living beings and hence they tend to accumulate high level of iron from the surrounding environments (Kanakaraju et al., 2008). With the maximum level of Fe in Cattle Egret and minimum in House Crow the values were comparable to the Fe level (4823.76 ± 8.99 µg/g) detected by Bravo et al. (2005) in faeces of Black Vulture. Mn is regulated in birds primarily by excretion in the faeces, however hens also excrete Mn in eggs. Sub-lethal

exposure of manganese to avian embryos have been shown to cause the teratogenic effects such as twisted limbs, neck defects, hemorrhage, and micromelia (Hashmi et al., 2013). No trophic level relationships have been found in a number of studies for Mn but Horai et al (2007) suggested the distribution of Mn in body of birds affected by diet. Sodium an essential mineral element is a recognized beneficial element. A large number of body processes depend on the presence of Na ions. The maximum level of Ni was found in Cattle Egret and the values were comparable to the level of Ni (15.19 ± 1.33 µg/g) in faeces of Black Vulture as observed by Bravo et al (2005). Above threshold level, Ni is seen to affect the respiratory system of birds, causing asthma, as well as birth defects, vomiting, and damage to DNA (Van wyk et al., 2001). Lead is the heaviest non-radioactive element and if ingested, it is poisonous to animals as well as birds. The maximum level of lead was observed in Common Myna followed by Cattle Egret in present studies. These results are supported by the studies of Burger and Gochfeld (1997) indicating that Cattle Egrets feed on insects on road where Pb deposition is likely to be high. Pb and Cd have no documented role in living organisms and have been associated with breeding failure in some egret and heron species (Burger, 1993). Birds exposed to Pb also showed decreased body weight and reproductive impairment (Hashmi et al., 2012). Bravo et al (2005) reported Zn level to be 202.57 ± 1.65 µg/g in faeces of Black Vulture (Coragyps atratus). Dewar et al (1983) experimentally poisoned chickens and reported lesions of the exocrine pancreas including dilation of acinar lumina, cytoplasmic vacuolation, cytoplasmic globule formation, necrosis, numerous mitotic figures and interparenchymal fibrosis. Differences in Zn concentrations in birds have been attributed to differences in diet and bioavailability (Adendorff, 1993). Thus justifying the differences in concentration of Zn in two omnivorous species i,e. Common Myna (maximum) and House Crow (minimum).

Besides these, some metals like K, Mg, Na, P and S are recognized as essential nutrients in animal nutrition and no toxic effect of these metals have been recorded in birds till date. The toxicity effects of sulphur are indirect due to acute exposure to H_2S gas with much higher concentrations (>200 ppm) and include pulmonary oedema, respiratory arrest, coma, and death in birds (Durand, 2007).

Species specific variations and toxicity of heavy metals

The significant interspecific differences in level of all the metals except K, Mg and Na were revealed in omnivorous, granivorous, and insectivorous birds. In present studies Cattle Egret was reported to excrete maximum level of As, Cd, Fe, Mn, Cr, Ni and Blue Rock Pigeon excreted highest level of Cu, Mg, P, S. On the other hand House Crow excreted highest level of B, K, Na and Common Myna excreted maximum level of Ca, Pb, Zn. Specific differences in the levels of accumulation of toxic elements at similar pollution levels are explained primarily by different specific peculiarities of the diets (Lebedeva, 1997). The animals of higher trophic levels accumulate some metals more intensively. Trophic-level relationships have been reported for a range of species and for a number of contaminants (Burger, 2002). Such relationships may be attributed to the bioaccumulation of different metals in food items of insectivorous and omnivorous species but not of granivorous species i,e. Blue Rock Pigeon. This may be because the amount of metal excreted does not reflect its amount accumulated within tissues; however it only indicates the extent of exposure of particular metal to birds. Furthermore, besides feeding several physiological and biological processes like species diet pattern and types, body condition, age, growth, moulting and reproduction may affect in the accumulation of metals in the tissues (Hashmi et al., 2013). Cd is known to accumulate with age and sex (Barbieri et al., 2007), as more accumulation in males than females and juveniles of Puffinus gravis was observed. Markowski et al. (2013) reported lead level in female bones 10 times higher than the males. As in present studies, the excreta were collected from the birds of crop field area with different sex and age group, so this may be another factor for species specific variations in the concentration of metals.

The presence of As, Cd, B and Pb metals above the normal range in birds reveals the high exposure to heavy metal contamination in their environment and may pose a threat to these species. Some earlier reports revealed that the concentrations of heavy metals in exposed birds above the threshold level but insufficient to cause direct mortality may result in behavioral changes, reproductive dysfunction, and increased chances of disease. 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 (Dauwe et al., 2004). Moreover, heavy metals are toxic because they may have cumulative deleterious effects that can cause chronic degenerative changes. Another important observation from this study was that the single bird species, Cattle Egret, excreting highest level of six metals i.e. As, Cd, Cr, Fe, Mn and Ni at a time than other four species is of more concern. Because the cumulative effect of heavy metals load can be fatal for single organism.

Thus it can be concluded from the present studies that presence of heavy metals in faecal pellets of all the five bird species make excreta a suitable indicator for the assessment of heavy metal contamination in wild birds without harming them. Further the significant variations in metal level among the species throw light on the fact that there are significant fluctuations in the level of contamination of the environment where these birds live. Evidently,

regular monitoring of heavy metal accumulation in wild bird populations on territories with different extents of pollution is necessary for a more complete characterization of the environmental quality.

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