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Estimation of heavy metal residues in excreta of spotted owlet (*Athene brama*) and barn owl (*Tyto alba*) from agro ecosystems of Punjab

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Abstract
The present study deals with the determination of different metals by using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICAP-AES) in the excreta samples of Spotted Owlet and Barn Owl collected from three different locations of Punjab. The results showed the presence of As, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S and Zn metals in excreta samples. Out of these elements As, B, Cd and Pb are included in the category of non-essential and toxic elements. In excreta of Spotted Owlet, level of As (0.78 – 3.35 ppm) was higher than the normal range. The metal Pb (9.28 – 29.20 ppm) and B (39.43 – 65.40 ppm) were found to be in toxic range in the samples collected from location I and II. In excreta of Barn Owl, level of As (1.99 ppm) was in high range whereas Pb (25.91 ppm) and B (19.20 ppm) were in toxic range. Cd (0.42 – 0.78 ppm) was present within the normal range in samples of both the owl species. Level of other metals like Cr, Ca, Fe, K, Mg, Mn, Ni, P, Zn was also higher than the normal range. Samples of Spotted Owlet had comparatively high level of heavy metals than the samples of Barn Owl.

Keywords: Spotted owlet, barn owl, heavy metals, agroecosystem, excreta

Introduction
Metals play an important role in biological processes but excess amount of these elements may cause chronic toxicity. Living birds are delicate to direct and indirect environmental effects and they can be considered as best bioindicators of metal contamination because they are visible, they have large geographic distribution area, feed at various trophic levels and many are long lived [1, 2]. Exposure to high levels of heavy metals through any means can have toxic effects on avian species influencing their hormone and respiratory systems, reproduction and migration [3]. Birds are exposed to heavy metal from drinking-water contamination, high ambient air metal concentrations near emission sources, or intake via the food [4]. The rate of heavy metal absorption varies depending on species physiology, metal properties, and bio-availability in the environment. They can rid the body of heavy metals by depositing them in the uropygial gland, salt gland or through the faeces [5]. A large portion of metals are excreted, thus faeces become appropriate source to assess the level of contamination [6]. Bird excrements have been suggested as useful non-destructive indicators of metal contamination in bird's diet [7, 8] and provide information about the metals which were absorbed and excreted [9]. Owls are the birds of prey, present at higher trophic levels which mostly prey on small mammals, insects, small birds and sometimes fish. Predatory birds attract the attention of ecotoxicologists due to their vulnerability, which is related firstly to the low density of their natural populations and secondly to their position at the top of trophic chains. Like other birds of prey, Spotted Owlet and Barn Owl are also adversely affected by indiscriminate use of pollutants and heavy metals in modern agriculture [10]. The population of these birds therefore appears to be declining in intensively cultivated areas like Punjab. So the present study was planned to analyze the heavy metal contamination in excreta of Spotted Owlet and Barn Owl inhabiting agroecosystems of Punjab.

Materials and methods
Dry excreta samples of Spotted Owlet and Barn Owl were collected from three locations i,e. location I- village Baranhara present near Buddha Nullah and location II- village Ladhowal present near Sutlej river and location III- field areas of Punjab Agricultural University of

district Ludhiana. Collection was done from the roosting and nesting sites of the selected species. 0.5 g of dry excreta samples was taken. Digestion was done with diacid mixture of HNO_3 and $HClO_4$. Then it was placed on hot plate for approximately three hours. The final volume was made 25 ml with distilled water and then solution was filtered. The digested samples were analysed for its elemental composition by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICAP-AES) in the Department of Soil Sciences, PAU, Ludhiana. Statistical analysis was done by using ANOVA. The observed values were compared with standard values of different metals for avian species by Wisconsin Veterinary Diagnostic Laboratory (WVDL), Toxicology centre, United States.

Results and Discussion

The concentration of different heavy metals was analyzed in the excreta of Spotted Owlet and Barn Owl. The metals detected in excreta samples of selected species were As, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, and Zn (Table 1). Out of these elements some heavy metals like As, B, Cd, Pb were reported to be toxic to birds and mammals [11]. Other elements like Fe, Cr, Cu, Ni, Zn, Mn are essential elements needed in body to perform some specific functions. But all these essential elements may also become toxic when present above threshold levels.

In excreta samples of Spotted Owlet the concentration of As was 3.35 ± 0.88, 2.11 ±0.24 and 1.78 ± 0.52 ppm at location I, II and III respectively. The concentration recorded was higher than the normal range of As. Chung *et al*. [12] reported that drinking water, food, occupational environment and industrial processes are routes that have been found to be the cause of As exposure. In case of B, the concentration level observed in the excreta was 65.40 ± 24.87, 63.43 ± 20.75 and 39.43 ±7.70 ppm at location I, II and III respectively. The level of B was observed to be highly toxic in all the three locations as it was much higher than the normal range. Boron compounds have moderate acute toxicity and the symptoms include depression, ataxia, congestion, convulsions and weight loss [5]. The level of Cd detected in excreta was 0.78 ± 0.15 at location I, 0.56 ± 0.05 at location II and 0.42 ± 0.07 at location III. The concentration of Cd recorded from all the locations was within the normal range may be due to the absence of industries nearby, which are considered as main source of cadmium release into the environment. The concentration of Pb recorded in the excreta was 29.20 ± 4.15, 22.28 ± 0.44 and 9.28 ± 0.72 at location I, II and III respectively. The concentration recorded from location I and II was observed to be toxic when compared with the normal range but the concentration from location III was not toxic. According to Gushit *et al*. [13] avian community from all the feeding guilds accumulate high amount of lead except the nectarivorous species. Birds exposed to Pb are reported to have reproductive impairment and decreased body weight [14]. The concentration level of calcium in excreta samples of Spotted Owlet was detected to be 20805.00 ± 3410.84, 14891.67 ± 552.19 and 1558.33 ± 216.61 ppm from location I, II and III respectively i,e. higher than the normal recommended range. Calcium above required level can cause calcification of kidneys, eggs and proventriculus and make chicks unable to hatch [5]. The minimum concentration was detected in samples of location III and maximum in samples from location I. The concentration level of Cr (ppm) in excreta samples of Spotted Owlet was detected to be 12.05 ± 3.28, 6.52 ± 0.34 and 3.52 ± 0.92 ppm from location I, II and III respectively. The minimum concentration was detected in samples of location III and maximum in samples from location I, but all the values were higher than normal range. The chromium produces adverse effects on the embryonic development and hatching success of nestling birds [15]. The concentration level of Cu (ppm) in excreta samples of Spotted Owlet was detected to be 26.71 ± 5.77, 54.00 ± 15.91 and 12.66 ± 1.46 ppm from location I, II and III respectively. Concentration recorded from location I and II was higher than the normal range whereas from location III was in between normal range. Copper is widely used in industries and agriculture and due to this Cu quantities in the environment have increased. Concentration of iron in excreta samples was detected as 5356.00 ± 1424.00, 2248.33 ± 54.41 and 646.67 ± 51.75 ppm from location I, II and III respectively. Level was higher at location I and II than III. The level of K in excreta samples of Spotted Owlet was detected to be 17191.67 ± 879.57, 26258.33 ± 795.93 and 6506.67 ± 668.36 ppm from location I, II and III respectively. The concentration level of Mg (ppm) in excreta samples of Spotted Owlet was detected to be 3448.00 ± 43.55, 3133.00 ± 467.35 and 1439.67 ± 47.70 ppm from location I, II and III respectively. The level of Mn in excreta samples of Spotted Owlet was detected to be 165.20 ± 41.20, 99.80 ± 4.56 and 45.80 ± 3.76 ppm from location I, II and III respectively. The minimum concentration was detected in samples of location III and maximum in samples from location II. Manganese in high concentrations effects on birds may include stunted growth, twisting of limbs, changes in behaviour and anaemia [16]. The sodium concentration in excreta samples of Spotted Owlet was detected to be 3436.33 ± 566.39, 4162.00 ± 359.93 and 1798.67 ± 175.00 ppm from location I, II and III respectively. The concentration of nickel in excreta samples of Spotted Owlet was detected to be 9.64 ± 2.36, 6.02 ± 0.88 and 3.02 ± 0.28 ppm from location I, II and III respectively. The minimum concentration was detected in samples of location III and maximum in samples from location I. Substantial quantity of the Ni from the environment when ingested find their way into the bone, lung, kidney, liver, brain and endocrine gland, also detected in breast milk, saliva, nails hair [13]. The concentration of Phosphorus in excreta samples of Spotted Owlet was detected to be 13246.67 ± 2465.48, 16458.33 ± 222.15 and 5591.67 ± 963.16 ppm from location I, II and III respectively. The minimum concentration was detected in samples of location III and maximum in samples from location I. The concentration of sulphur in excreta samples of Spotted Owlet was detected to be 10451.67 ± 2779.82, 10723.00 ± 1188.35 and 2423.00 ± 303.12 ppm from location I, II and III respectively. The minimum concentration was detected in samples of location III and maximum in samples from location II. The concentration of Zinc in excreta samples of Spotted Owlet was detected to be 189.33 ± 38.54, 289.83 ± 34.79 and 48.83 ± 10.90 ppm from location I, II and III respectively. The minimum concentration was detected in samples from location III and maximum in samples from location II. Zn has an important role in activation of enzymes and the regulation of gene expression but its higher concentration of Zn may impair physiological functions of birds, as well as contributing to decline in species populations [17].

Table 1: Level of different metals in excreta of Spotted Owlet from different locations

Elements	Location I	Location II	Location III	Normal range	Toxic range
	Baranhara	Ladhowal	PAU		
Arsenic*	3.35 ± 0.88	2.11 ± 0.24	0.78 ±0.12	0.01-0.2	5-10
Boron	65.40 ± 24.87	63.43 ± 20.75	39.43 ± 7.70	0.13-5	6-89
Calcium*	20805.00 ± 3410.84	14891.67 ± 552.19	1558.33 ± 216.61	170-600	-
Cadmium	0.78 ± 0.15	0.56 ± 0.05	0.42 ± 0.07	0.02-1.5	70-140
Chromium	12.05 ± 3.28	6.52 ± 0.34	3.52 ± 0.92	0.05-0.4	19-170
Copper	26.71 ± 5.77	54.00 ± 15.91	12.66 ± 1.46	3-15	275-800
Iron*	5356.00 ± 1424.00	2248.33 ± 54.41	646.67 ± 51.75	45-300	>8000
Potassium*	17191.67 ± 879.57	26258.33 ± 795.93	6506.67 ± 668.36	1960-2600	-
Magnesium*	3448.00 ± 43.55	3133.00 ± 467.35	1439.67 ± 47.70	15-36	30-50
Manganese*	165.20 ± 41.20	99.80 ± 4.56	45.80 ± 3.76	2-4	>9
Sodium*	3436.33 ± 566.39	4162.00 ± 359.93	1798.67 ± 175.00	2800-3750	3500-6000
Nickel*	9.64 ± 2.36	6.02 ± 0.88	3.02 ± 0.28	0.04-0.13	10-12
Phosphorus*	13246.67 ± 2465.48	16458.33 ± 222.15	5591.67 ± 963.16	110-150	190-200
Lead*	29.20 ± 4.15	22.28 ± 0.44	9.28 ± 0.72	0.01-1	8-1600
Sulphur*	10451.67 ± 2779.82	10723.00 ± 1188.35	2423.00 ± 303.12	-	-
Zinc*	189.33 ± 38.54	289.83 ± 34.79	48.83 ± 10.90	22-40	300-800

The data is represented as mean ± standard error of three samples from each site.
* Significantly differ in different sites, $P \leq 0.05$.

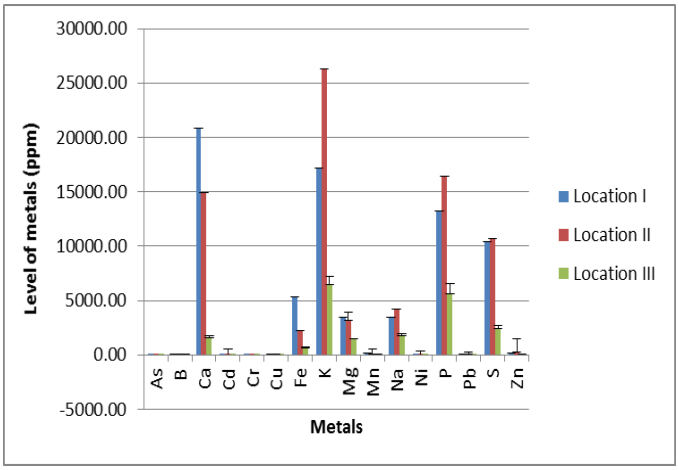

Fig 1: Level of different metals in excreta of Spotted Owlet from different locations

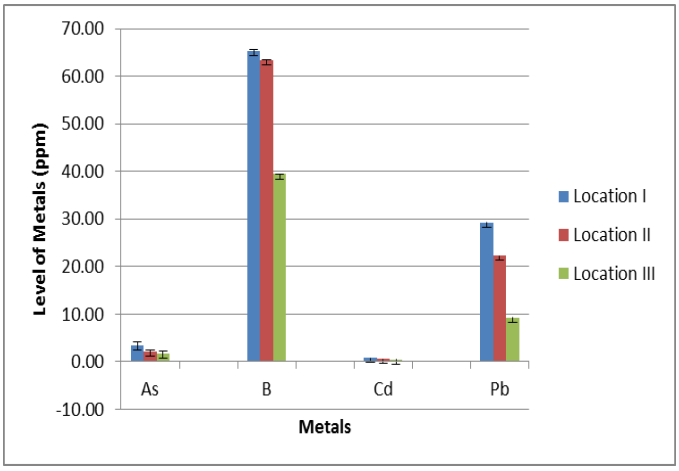

Fig 2: Level of heavy metals in excreta of Spotted Owlet from different locations

Barn Owl excreta was collected only from location II i.e. village Ladhowal. As it is nocturnal and large bird of prey so it was difficult to locate its roosting or nesting sites. In excreta samples of Barn Owl the concentration of As was 1.99 ± 0.13 ppm. The concentration recorded was higher than the normal range of As but lower than the toxic range. In case of B, the concentration level observed in the excreta was 19.20 ± 3.39 ppm. The level of B was observed to be higher than the normal range. The level of Cd i.e. 0.53 ± 0.14 ppm was detected to be within normal range in Barn Owl sample. The concentration of Pb recorded in the excreta was 25.91 ± 1.13 ppm i.e. in toxic range. The level of Ca and Cr were found to be 10958.00 ± 2126.87 and 6.47 ± 0.13 ppm respectively. The level of Cu i,e. 10.92 ± 0.25 ppm was reported in normal range. The level of Fe and K were found to be 1683.67 ± 398.50 and 7505.00 ± 227.72 ppm respectively, which were above the normal range. The level of Mg and Mn were found to be 2608.33 ± 95.36 and 84.30 ± 12.42 ppm respectively which were reported under toxic range. The level of Na i.e. 499.00 ± 15.31 ppm observed in Barn Owl samples was less than the normal range. The level of Ni (6.320 ±0.035 ppm) was found to be higher than the normal range. The level of P i.e. 4317.33 ± 259.04 ppm was found to be toxic as in samples of Spotted Owlet. The level of S was found to be 3547.67 ± 226 ppm. The level of Zn was found to be 111.07 ± 0.75 ppm i.e. in high range like samples of Spotted Owlet.

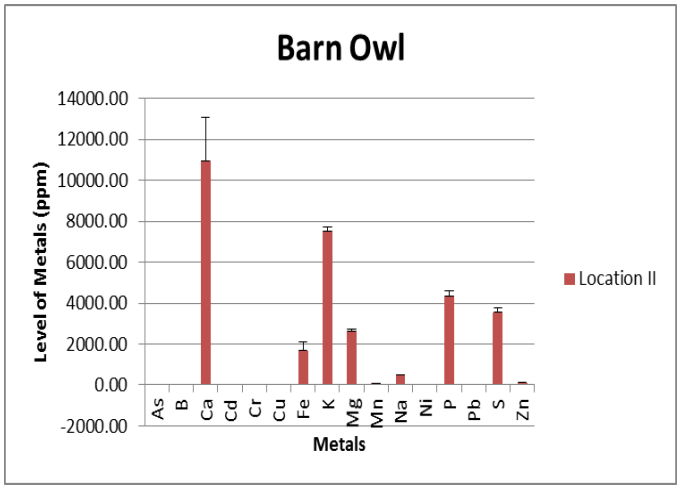

Fig 3: Level of different metals in excreta of Barn Owl from location II

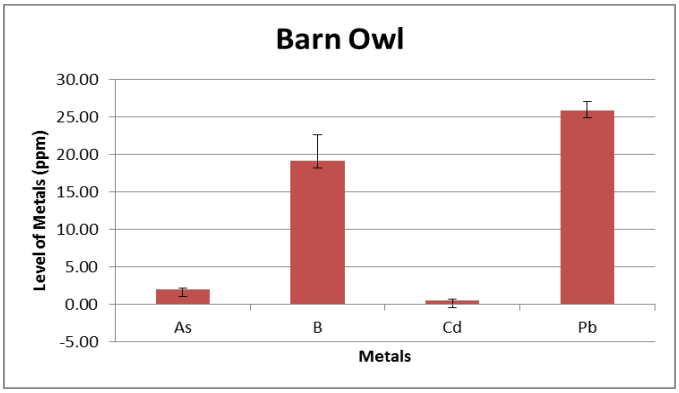

Fig 4: Level of heavy metals in excreta of Barn Owl from location II

In the present studies, there was a significant difference in the level of different metals among selected locations in the excreta of Spotted Owlet. Concentration Level of As was high, B and Pb were found to be toxic and Cd was under normal range in the excreta samples of Spotted Owlet among all the three locations from the results. High level of B may be due to application of borax in agrifields at the time of fruiting of trees in field area [18]. It was reported that Location I had the maximum concentration of heavy metals than location II and III. In case of Barn Owl, heavy metals like Pb was found to be toxic, level of As and B was high whereas Cd was in between the normal range. Level of other metals like Cr, Ca, Fe, K, Mg, Mn, Ni, P, Zn was also higher than the normal range this may be related to presence of Sutlej river near the village. Other reasons could also be there like pollutants and pesticides are widely distributed now days. Main source of pollution include excessive use of fertilizers, herbicides, pesticides, toxic substance spills from refineries [19]. Some earlier reports revealed that the concentrations of heavy metals in exposed birds above the threshold level were insufficient to cause direct mortality but may result in reproductive dysfunction, increased chances of disease and behavioural changes [20]. According to Chaplygina and Yuzyk [21], flycatchers had been known to accumulate excess amount of cadmium and lead in their body that may cause physiological dysfunctions of their organs, pathological changes, decrease in hemoglobin levels, reduction of clutch size and elevated embryonic and bird mortality. Kertesz and Fancsi [15] reported that during the embryonic development chromium show adverse effects and also reduce hatching success in mallard. Dove and Haydon [22] reported that copper containing dietary supplements changes the tissue fatty acid pattern and also alters lipid metabolism in swine. The deficiency of sodium can cause weight loss, slow growth, excessive fluid excretion and fatigue but high dose of salt in birds may lead to mild to moderate kidney dysfunction [23]. Many workers have found high level of Ni concentrations in the passerine birds [20, 24]. The inhalation of dust containing large amount of Sulphur can causes eye or skin irritation. The study conducted by Kazacos and Van Vleet [25] on Pekin ducklings reported the Zn toxicity in interstitial fibrosis, atropic acinar cells, and the formation of duct-like structures. On comparison of samples of Spotted Owlet and Barn Owl from location II, a significant difference was observed among different metals. Level was comparatively high in samples of Spotted Owlet because samples were collected from residential area whereas sample of Barn Owl were collected from University Seed Farm, where ideal agricultural practices are being followed.

Conclusion

It can be concluded that Location I had the maximum concentration of heavy metals than location II and III. Location I is considered as most contaminated site which may be related to the presence of Buddha Nullah flowing inside the village. High level of As and Pb in location I and II may be due to contamination in Buddha Nullah and Sutlej river flowing near the villages. The location III had least concentration of metals (almost within the normal range) which may be due to controlled conditions followed by PAU and a clean environment than other locations. Variations in heavy metal concentration among the species throw light on the fact that there are significant fluctuations in the level of contamination of the environment where these birds lives and feeds. Further detailed studies on the feeding guilds of these birds may provide a useful guide in determining the sources of the pollutants in the environment.

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