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Biomonitoring of heavy metal signatures in urbanized contaminated ecosystems of Southern India: A case study using raptor, *Athene brama*

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Abstract

Background: Heavy metals remain tenacious in the environment which further leads to emerging bioaccumulation, rising through the food chain. Birds are great environmental sentinels that can spot even the smallest changes. The aim of this study was to determine the quantity of heavy metals, specifically Ni, copper (Cu), aluminum (Al), titanium (Ti), and Se, in the feathers, liver, kidney, muscle, and bones of spotted owlets (*Athene brama*), which were opportunistically collected from a variety of contaminated ecosystems in the urbanized landscapes of Visakhapatnam on India's East Coast.

Methods: Opportunistic sample collection was done followed by necropsy. Following standard protocols, organic samples were digested by adding 10 mL HNO_3, 5 mL of $HClO_4$, and 2 mL of H_2O_2 to eliminate the excess organic matter.

Results: Based on the GIS data, raptors were shown to occur in production landscapes and mostly in urban spaces, and currently, are under several anthropogenic threats. The findings showed that the sequence of heavy metal concentrations was Ti > Al > Cu > Ni > Se, but the tissues of birds in which metals accumulated are in the following order: liver > kidney > bones > muscle. The tissues of *A. brama* showed the signs of significant concentrations of Ti and Al. The results obtained were analyzed using Kruskal-Wallis non-parametric test.

Conclusion: Heavy metal studies on various matrices of the environment have been performed over time with certain metals either neglected or not being documented properly. The main finding of this study is the first report of Ti and Al in bird species from India as there is a lacuna in this context.

Keywords: Bio-accumulation, Feathers, Titanium, Birds, Ecosystem

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Introduction

Avian taxa are characterized primarily by their distinct ecological feature of roosting. A major portion of the day is spent by birds on their roosts while carrying out activities like preening, resting, hiding from predators etc (1). Spotted owlet (*Athene brama*) are nocturnal birds of prey who feed on small rodents, insects, small birds, etc. The first report on using *Aves* as a biomonitor for monitoring and accessing the health of an environment was carried out in the 1960s (2). Biodiversity and human health are continuously being affected due to heavy metal exposure, which arises from either natural or man-made causes. As birds are vulnerable to changes in the environment, hence, they have been reported to be suitable bio-indicators (3). Tertiary consumers help in biomonitoring the health of an ecosystem in both food chain and food web (4-7). Putting together significant information to detect the condition of the environment is largely dependent on the key role birds play (8). The abundant bioavailability of certain heavy metals like zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb), and chromium arising in connection to agricultural runoff, and industrial outputs are responsible for the bioaccumulation of metals in living organisms occurring through different routes of entry and largely influencing the health of the particular organism. The pathway of magnification of these metals and their transfer through different trophic levels are eased by the top predators of the food chain like raptors (9). As these metals possess characteristics of biomagnification, hence, several studies have concluded that certain elements like arsenic (As) and mercury (Hg) are bioavailable and they bio-magnify through the food-chain and threaten the overall health of living beings (7-10). The studies had reported that for certain species, long-term exposure to the pollutants or contaminants is because of their biological habitat preferences, which when subjected to analysis would have otherwise generated a great amount of information. The assessment and analysis of the internal organs of raptors (tissues, bones, etc) allow for estimating the quality of the environment in which the bird is in or perhaps its habitat. It also gives an idea about the possible risk of exposure to the species being studied (11). Muralidharan et al reported that birds are useful tools for evaluating environmental pollution extent (12). Moreover, birds are easily accessible, widely spread across different ecosystems and show changes to toxicants (13), forage over a large biogeographical area, and can reflect the current state of the environment they belong in (7). Just like wild animals, birds such as waterfowl and raptors are susceptible to death because of poisoning which may also occur through game hunting (14). Raptors such as buzzards, *Bubo bengalensis* (Indian Eagle Owl), and little owls that generally forage on different varieties of prey can be useful in extracting information about bioaccumulation and environmental biomonitoring (8,15). Raptors such as the Indian Eagle Owl (*B. bengalensis)* have often been utilized as a bio-monitor across the world for ecological health monitoring (7,16).

Given the causes of anthropogenic point and non-point sources of discharging toxic elements into the environment, the accumulation of heavy metals is a continuous process that takes place through prolonged exposure or accumulation. In some cases, they can even change into a more toxic form occurring through biochemical processes (10). Human activities are solely responsible for the degradation of coastal and aquatic environments (17). Certain elements like iron (Fe), Zn, Mo, cobalt (Co), manganese (Mn), molybdenum (Mo), and Cu are necessary for the development of all organisms but can be toxic if present in unrestrained quantities (18). Toxicity signatures that many metals exhibit has shown to disrupt the performance of important bodily functions like central nervous system (CNS) (19). While certain other toxic elements like Cd and vanadium (Va) have been reported to show useful and beneficial properties when being subjected to various conditions (17). According to the study of Biswas et al (6), heavy metals accumulate in detoxifying organs, and certain ways through which birds get rid of metals are excretion, moulting or depositing in egg shells. According to the study of Grúz et al (10), the collection of feathers from birds is a non-invasive technique and it represents the amount of metal burden internally, as well as externally through moulting (20). There are many adverse effects associated with metal accumulation in birds. Apart from changes in behavioural patterns, major deformities also affect a bird's reproductive health (7). Hence, monitoring of these toxic elements provides a complete overview of the bird's environment apart from providing species-specific information (21).

Lacuna exists in information and data available on heavy metal accumulation in birds in the Indian context (7,21-25). There is no study conducted on this subject in Andhra Pradesh.

Visakhapatnam, Andhra Pradesh, is a hub for industrial activities that include ports, Visakhapatnam Steel Plant, National Thermal Power Corporation Limited (NTPC), and several other small and large-scale industries (paint, textile, battery, cement, pharmaceuticals, etc). It is subdivided into many other districts like Visakhapatnam urban (the present study area) and rural areas. The impact of urbanization in this region has resulted in the degradation of biodiversity in the past decade. The simplification of natural habitats into production landscapes, sewage dumps, and wastelands are by far the major anthropogenic factors responsible for diminishing bird populations. Spotted owlet (*Athene brama*), family *Strigidae* are small owls that feed on small mammals, insects, etc, and roost on human-dominated landscapes. They are often found in agricultural fields, farmlands, open areas, and city spaces. Due to their foraging habits, they are very likely to be exposed to diets that may be contaminated because of the environment they sustain in. The content of metal bio-accumulated in such birds are useful in providing an idea about their ecological habitat and the foraging environment they are situated in. The present study aimed to investigate heavy metals (Ni, Cu, Al, Ti, Se) in their internal organs (kidney, liver, muscle, bone) and feathers (primary, secondary and breast feathers) to collect data regarding the current circumstances of their foraging environment that would give a clearer understanding of the ecosystem health through implication of GIS and satellite-based data of this bird occurred on most of the urban dominated lands, including mined lands, which are continuously under the threat of constant urbanization.

Materials and Methods

Spatial distribution and habitat of spotted owlet (Athene brama)

Breeding through tropical Asia, the spotted owlet (*A. brama*) is a small-sized owl that usually inhabits Southeast Asia and mainland of India. Some habitats preferred by these birds include agricultural fields, pastures, farmlands, and urban landscapes including parks and gardens. Over the time span of the past decade since urbanization escalated, these owls have adapted well to cities. Their roosts consist of small groups, often found in tree cavities or hollows, rock creeks or perhaps in human habitats like residential complexes and buildings, etc.

Figure 1 shows the sightings (n = 140) of spotted owlet (*A. brama*) from various corners of the Visakhapatnam district that have been gathered on the basis of data provided by local birders, technically combined by (26) and downloaded from the database of Global Biodiversity Information Facility (GBIF) (11). In order to understand their distribution across the human-dominated landscapes, a spatial distribution map of the species was prepared using QGIS 3.16.8 Desktop. The map was prepared with the objective to show the land use

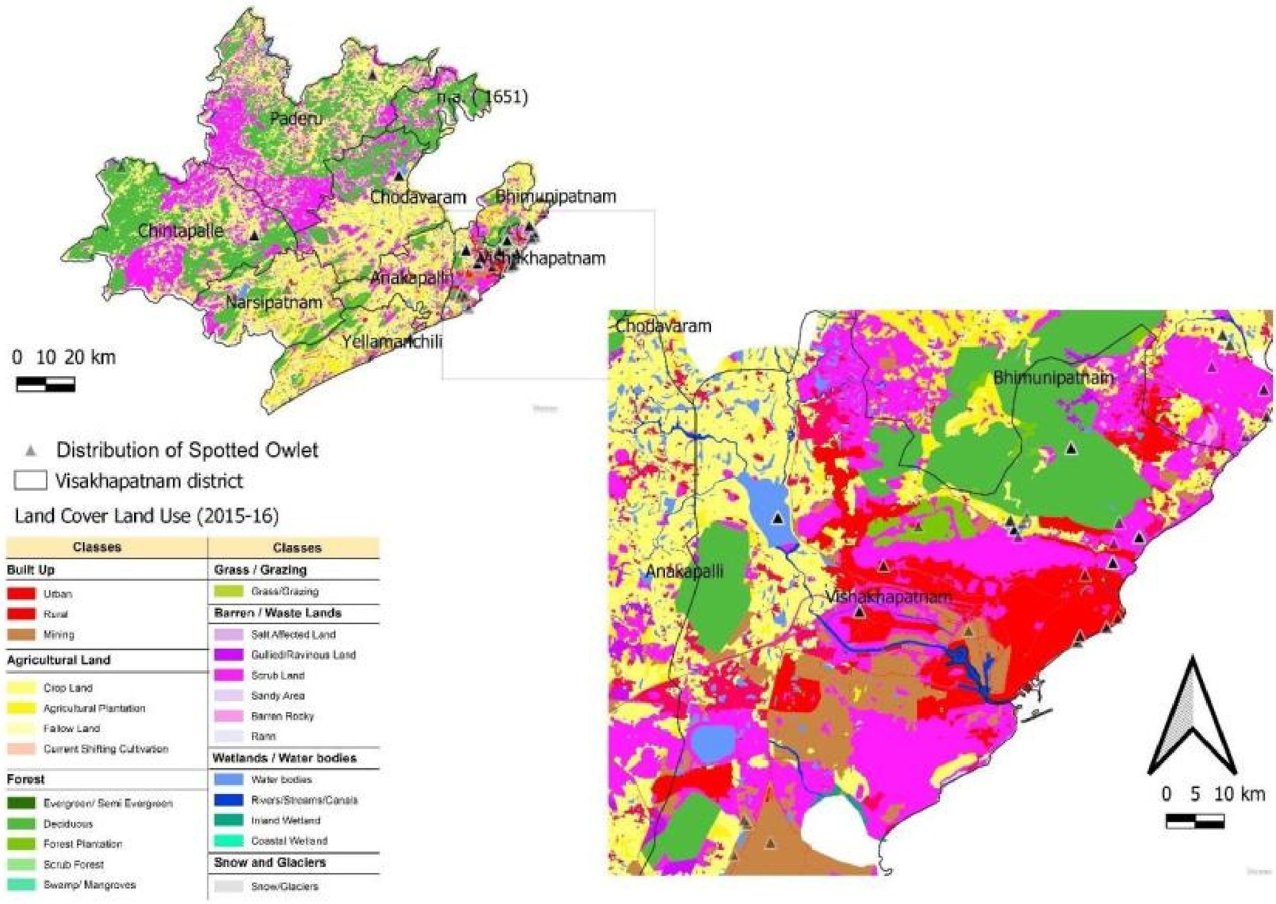

Figure 1. Maximum recorded sightings of Spotted Owlet (*Athene brama*) from the district of Visakhapatnam (GBIF) over a backdrop of Land Cover Land Use (2015-16) of Visakhapatnam, data acquired from https://bhuvan-app1.nrsc.gov.in/.

and land cover map of Visakhapatnam (2015-2016 data accessed from (27) in the backdrop, thus, highlighting the number of sightings with respect to urban land utilization. Based on the map prepared through GIS and Bhuvan (satellite data), spotted owlet (*A. brama*) was observed mostly in urban-dominated spaces in Visakhapatnam including Gajuwaka, Steel Plant Area, Pedagantyada, Parvada, Bheemunipatnam, Kailasakona, Gopalapatnam, Simhachalam, and Kambalakonda. These areas were also the same areas which correlated with the areas from where specimens were collected for the present study.

Study area

Visakhapatnam is located on the South-Eastern coast of the country in the state of Andhra Pradesh (also known as Vizag) with a total geographical area of 11 161 km^2. It is a port city, and industrial hub, which shares its state boundary with other states *viz.* Tamil Nadu, Telangana and Orissa. With an undulating topography and a height above 4 to 50 m (above MSL), the Bay of Bengal is situated to its East, which borders the entire stretch of East Coast. Parts of Eastern Ghats ranges which are discontinuous hill ranges also run through Visakhapatnam where it is bounded by Simhachalam, Kambalakonda, and Kailash Konda hills. Since the city is located on the Bay of Bengal shores, the temperature and humidity of this area usually remain high.

In India, the Wildlife Protection Act of 1972 (Schedule IV) constitutes protection for all wild animals and birds and defying the law is a punishable offence. Hence, only random and opportunistic collection of morbid and dead birds was undertaken in the present study. The bird specimens were collected from various areas, as well as reserved forests. The permission was sought from the Divisional Forest Officer (DFO) and with the network of AP Forest Department and locals, the specimens were collected for a period of 2 years, from September 2018 to September 2020. The sample size of the present study was n = 36. The majority of samples were collected from colonies and urban spaces close to Simhachalam (including Kailash Konda, Kambalakonda), Gajuwaka and adjacent areas. Figure 2 represents the study area and sampling points from where the birds were collected.

Sample dissection

Following collection, the birds were transported to the lab in iceboxes to avoid decomposition. After thawing for 30 minutes, all samples were subjected to dissection. Following the removal of the breast feathers, the epidermis was gently cut open with a scalpel, and breast tissue was removed,

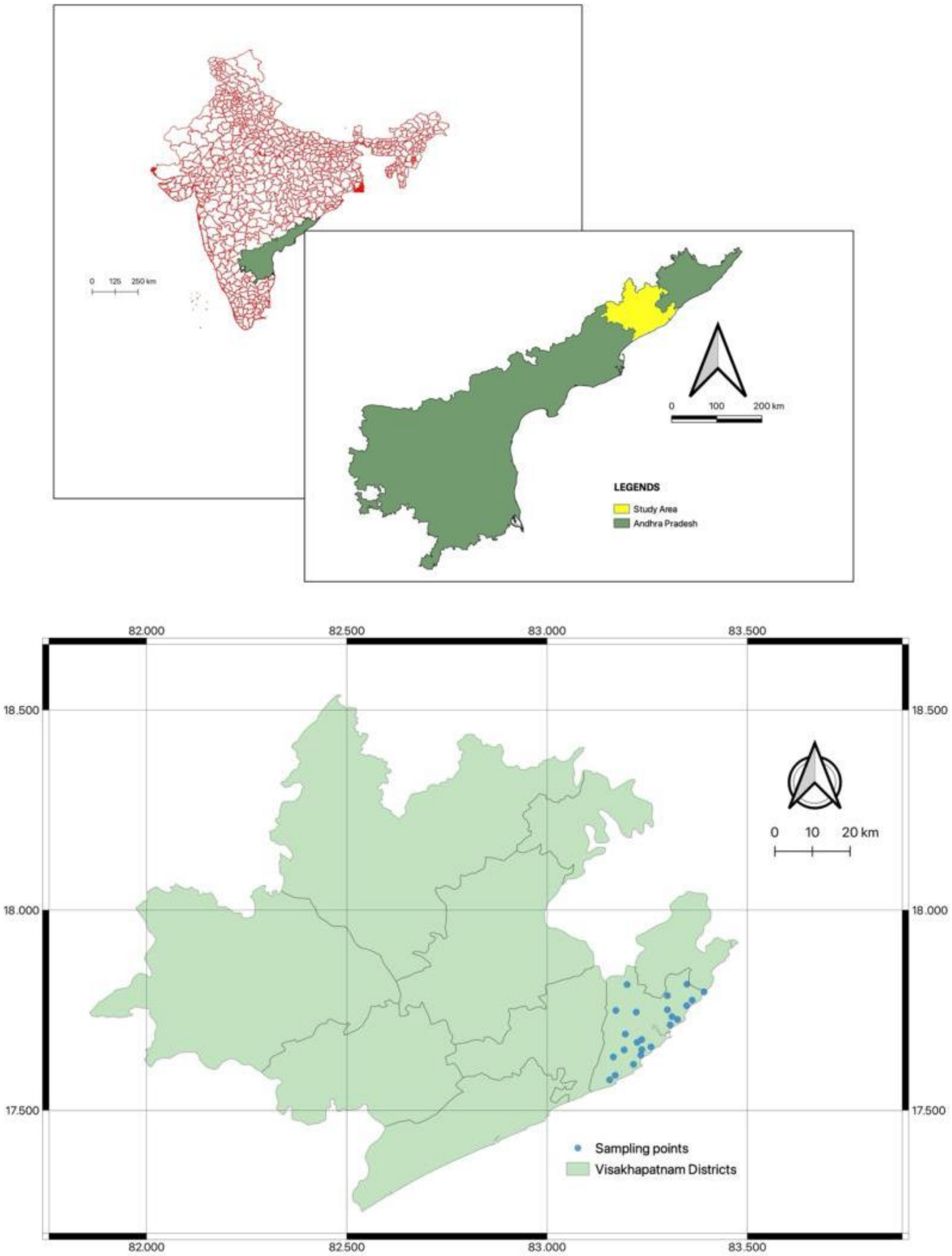

Figure 2. The study area map with the location of sampling points from different urban-dominated spaces of Visakhapatnam urban.

packed in aluminium foil, and labelled. The presence of any fluid, blood clots or exudates were observed. The visceral contents were removed using forceps and scissors and the organs and tissue (liver, kidney, breast tissue) were removed, labelled and wrapped in foil, and stored at -20ºC for further analysis.

Sample preparation, acid digestion, and elemental analysis

Decontamination and cleansing of all glass wares were done using MilliporeSigma Extran® MA 03 solution. The chemicals and reagents used for the study include nitric acid, (69% Emplura Merck), hydrogen peroxide H_2O_2 (30% Emplura Merck), and perchloric acid, 60% Emparta ACS), were purchased from Merck, Germany. After thawing, all tissues were hot air oven dried at 80ºC for 24 hours. Fine powder was made out of the tissues by drying and grinding them later with mortar and pistil. The process of acid digestion was performed according to the procedure outlined by Muralidharan et al (12). About 5 g of powdered sample was weighed and placed in Teflon beakers and to this, 10 mL of nitric acid (69% Emplura

Merck) was added and heated slowly on a hot plate. The heating was continued till a transparent solution obtained. Following this, 5 mL of perchloric acid * 60% Emparta ACS was added and again re-heated for 20 minutes. Afterwards, a more transparent mixture was obtained. Finally, hydrogen peroxide (30% Emplura Merck) was added to digest the excess organic content. Whatman No.1 filter paper was used to filter out coarse particles from the sample, and finally, millipore water (18.2 Ω, Elga PURE Waterlab) was used to increase volume to 25 mL. All the samples were stored in Tarson vials and refrigerated for further analysis. IC-PMS Inductively Coupled Plasma Mass Spectrometry (ICPMS; Agilent 770s, Agilent Technologies, Japan) was used to analyse the metals (Ni, Cu, Al, Ti, Se) and calibration standards by the National Institute of Standard Technology, NIST, U.S were used for each sample. All samples were run in triplicates.

Results

Totally, 36 individuals of spotted owlet (*A. brama*) were randomly collected dead or upon being dead and were included in this study. Heavy metal concentrations, namely Ti, Al, Cu, Ni, and Se were put together to take a glance at the varying levels of contamination between the tissues and feathers of the species. The results were expressed in dry weight, considering the fact that more consistency can be given to dry weight values (6,28). The accumulation pattern of the metals was found to follow the order Ti > Al > Cu > Ni > Se. The varying elemental characterization among the tissues and feathers of spotted owlet (*A. brama*) recorded in this study are represented in Table 1 and graphically shown in Figure 3. Figure 4 shows the concentration and accumulation of heavy metals (Ti, Al, Cu, Ni, and Se), which were observed in mean of all samples among organs namely liver, kidney, bone, and muscle of spotted owlet (*A. brama*), which followed the order liver > kidney > bones > muscle.

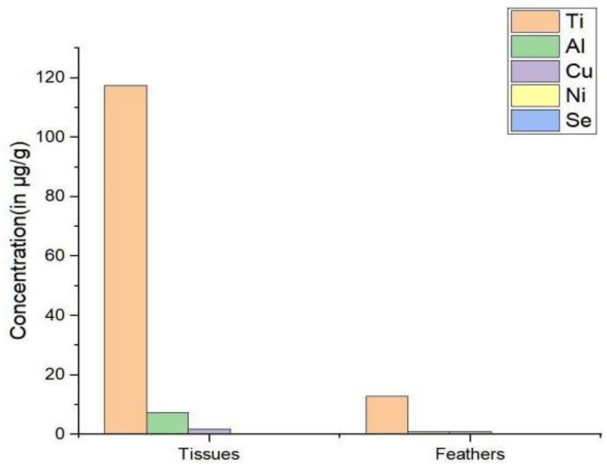

Figure 3. The concentration and accumulation of heavy metals (Ti, Al, Cu, Ni, and Se) observed in mean of all samples among the tissues and feathers of Spotted Owlet (Athene brama) expressed in µg/g.

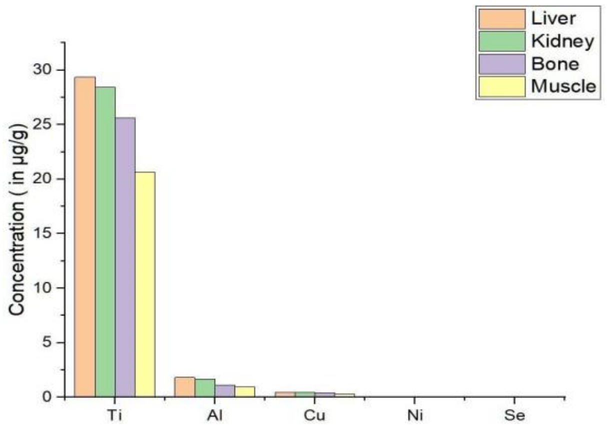

Figure 4. The concentration and accumulation of heavy metals (Ti, Al, Cu, Ni, and Se) observed among mean of all samples in various organs namely liver, kidney, bone, muscle of Spotted Owlet (Athene brama) expressed in µg/g.

Discussion

The heavy metal concentration directly reflected information on the food source of these birds and throw light on the environment they forage in. Sani et al reported that different geographical exposure, atmospheric deposition, or bird species' foraging patterns may account for variations in metal concentrations in different places (19). The results obtained for each heavy metal are discussed below:

Titanium

Among all the heavy metals analyzed in the present study, Ti had the highest concentration in liver (29.37 ± 6.43 µg/g) followed by kidney (28.41 ± 8.41 µg/g), bone (25.63 ± 5.45 µg/g), and muscle (20.67 ± 3.78 µg/g) of spotted owlet (*A. brama*). Titanium is a skin and eye irritant, and is also, associated with disrupting lung function, pleural disease, and it can lead to angina, thereby causing tightness in chest and breathing difficulties. As per literature, the first record of Ti in birds was reported by Ansara-Ross et al (29) in species of endangered grass owl from South Africa. The concentrations observed in the present study (118.18 ± 22.48 µg/g) were much higher than the values recorded in 2016 by Borghesi et al (17). However, the mean value of Titanium recorded in the present study (26.02 ± 7.12 µg/g) was in support of and correlated with that (27.5 ± 1.88 µg/g) reported by Ansara-Ross et al (29). They concluded that metals which have been frequently

Table 1. Mean concentration of heavy metals in tissues and feathers of spotted owlet (*Athene brama*) expressed in µg/g along with ± SE

Type of sample	Ti	Al	Cu	Ni	Se
Tissues	118.18 ± 22.48	7.33 ± 2.16	1.89 ± 0.70	0.06 ± 0.01	0.03 ± 0.01
Feathers	12.76 ± 3.08	0.97 ± 0.10	1.03 ± 0.72	0.04 ± 0.01	0.02 ± 0.01

examined include Pb, Cd, Cu, and Zn whereas inadequate data are available on metals like strontium (Sr), Va, antimony (Sb), and Ti (29).

Aluminium

The concentration of Al was recorded to be 1.81 ± 0.79 μg/g in liver, 1.65 ± 0.20 μg/g in kidney, 1.14 ± 0.98 μg/g in bone, and 0.94 ± 0.79 μg/g in muscle. Calcium homeostasis is severely affected by Aluminium impairment and it interferes with phosphorus metabolism as Scheuhammer (30) concluded that unfavourable effects of Aluminium can cause muscle weakness and retarded growth rate. Disruptive eggshell formation along with internal uterine bleeding has also been observed in pied flycatchers. The highest concentration of Al reported in the literature was reported 226 ± 515 μg/g in greylag goose from Atlantic Coast of France (31). The levels recorded in the present study (7.33 ± 2.16 μg/g) were lower than that recorded by other studies. There is a paucity of information on Al content in birds, which has been studied across the world.

Copper

The concentration of Cu recorded in the study was 0.47 ± 0.10 μg/g in liver, 0.44 ± 0.21 μg/g in kidney, 0.39 ± 0.21 μg/g in bone, and 0.28 ± 0.10 μg/g in muscle of spotted owlet. Cu has been reported by different authors worldwide and is a frequently investigated heavy metal. Venkatasalam et al reported Cu concentrations to be in the range of 6.88–73.58 μg/g in various species of birds studied across India (25). When compared to other studies, the present study recorded much lower levels of Cu.

Nickel

The concentration of Ni was reported to be 0.015 ± 0.01 μg/g in liver, 0.011 ± 0.01 μg/g in kidney, 0.008 ± 0.01 μg/g in bone, and 0.009 ± 0.001 μg/g in muscle. According to the study of Outridge and Scheuhammer (32), Ni concentrations greater than 10 μg/g (dry weight) in the kidney, and greater than 3 μg/g (dry weight) in the liver are toxic to wild birds. The levels reported in the present study are not indicative of any toxicity.

Selenium

When compared to other studies, the concentration of Se in the present study was found to be present in negligible concentrations.

Statistical analysis

Kruskal-Wallis ANOVA

The results of the present study were analyzed using Origin Pro 2021b SR2 Statistical software. To study the variation among samples, Kruskal-Wallis ANOVA test was used, the results indicated that the levels were not significantly different among the populations ($P > 0.05$).

Pearson's correlation coefficient

To investigate the relationship between two variables or to observe the similarities among two variables (in this case, metal accumulation by organs), Pearson's correlation coefficient was used by Origin Pro 2021b SR2 statistical software (Table 2), the results showed significant positive correlations between all the organs analysed ($r = 0.99$). According to the studies by Muralidharan et al and Sani et al, detoxifying organs like the liver and kidney of birds provides noteworthy and important information about contaminated environments and can act as suitable bioindicators (12,19).

Principal component analysis

Principal component analysis (PCA) is a predictive model for exploratory data analysis. In the PCA, PC1, and PC2 which were obtained, had explained 97.3% variance of the data. As shown in Figure 5, PC1 had positive loadings of all metals (Ti, Al, Se) and showed a strong relationship with higher concentrations in tissues indicating a similar point of origin which could be from industrial output or anthropogenic origin. The sampling areas are very closely situated to several small-scale industries like the paint and textile industry apart from slaughterhouses. The waste from slaughterhouses and these industries is very likely to get discharged in the nearby streams that connect the entire city which also includes the foraging habitat of these birds. In PC2 (Figure 5), negative loadings of Cu and Ni indicated that its source could be from natural origins like dust arising from wind-blown particles, sea spray, decaying vegetation, and forest fires. Simhachalam hills have frequently reported that forest fires and sea spray has been predominant factor in Visakhapatnam.

Conclusion

Over time, heavy metal research on diverse environmental matrices has been undertaken, with some metals either being overlooked or not being fully documented. There is a research lacuna in terms of establishing a baseline value for toxicity exerted by each heavy metal on birds in the Indian context and this study was also highlighted the need for it. While other authors across the world have notably contributed to determining toxic levels of certain elements like Pb, Sr, Cd, Cr, and Ni, there are few studies on other important elements like Ti, Al, and Sb. A fundamental and novel investigation of this study

Table 2. Correlational analysis of metals among the different organs of spotted owlet (*Athene brama*) evaluated in the present study

	Liver	Kidney	Bone	Muscle
Liver	1			
Kidney	0.999994	1		
Bone	0.999859	0.99991	1	
Muscle	0.999881	0.999927	0.999998	1

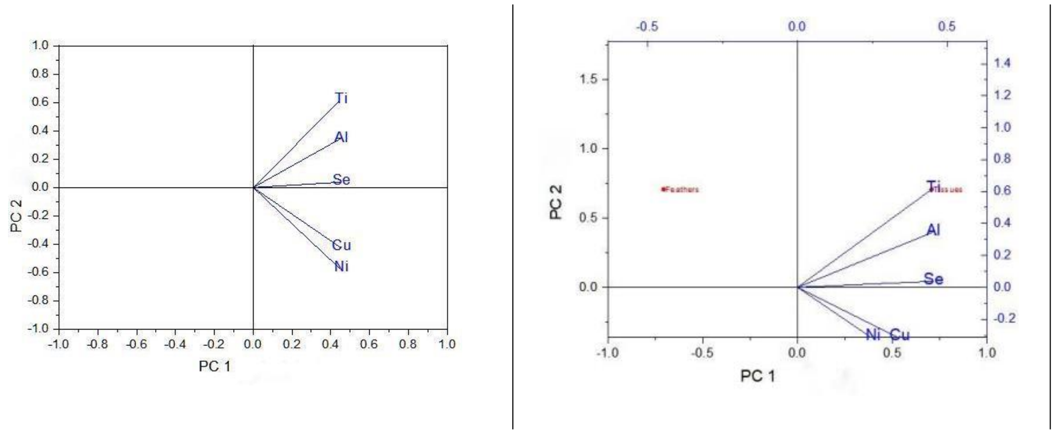

Figure 5. Principal component analysis, PC1 and PC2 with respect to the origin of metals.

revealed the findings of Titanium and Aluminium for the first time in bird species from India. Residential raptors like spotted owlet (*A. brama*) are not migratory and are openly distributed across landscapes and urban areas. The heavy metal values directly reflected information on the food source of these birds and throw light on the environment they forage in. Industrial units like slaughterhouses, paint, tanning, and textile industries along with large-scale industries like steel plants, and petroleum and cement industries mainly dispose Mn, Cu, Ni, Cr, Cd, Hg, and oxide titanium, Fe, and Al in the environment. The internal waterways of Visakhapatnam are connected and are a potential source for the inflow of toxicants into birds. Although in the absence of tissue, the non-invasive methods like analyzing feathers developed over the years in furnishing information about toxicants, extensive research to establish a baseline and toxic level value for heavy metals remains an existing gap. Being top-level predators, raptors are most susceptible to heavy metal biomagnification in the food chain. The present study concluded the strong presence of Ni, Cu, Al, Ti, and Se in internal organs like kidney, liver, muscle, bone, and feathers (primary, secondary and breast feathers) of *A. brama*. It also concluded that when compared to feathers, tissues in *A. brama* had accumulated more amounts of heavy metals but due to the paucity of data available on the levels of titanium and Al (considered toxic for birds), it was hard to establish if all together, these materials were responsible for the mortality in birds.

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Ethical issues
There were no ethical issues in the study as permission was sought from DFO and opportunistic random sampling was carried out.

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

Authors' contribution
Conceptualization: Sanchari Biswas.
Data curation: Sanchari Biswas.
Formal Analysis: Sanchari Biswas.
Funding acquisition: Ramakrishna Chinthala.
Investigation: Sanchari Biswas.
Methodology: Sanchari Biswas.
Project administration: Sanchari Biswas.
Resources: Sanchari Biswas.
Supervision: Ramakrishna Chinthala.
Validation: Ramakrishna Chinthala.
Visualization: Sanchari Biswas, Ramakrishna Chinthala.
Writing - original draft: Sanchari Biswas.
Writing - review & editing: Sanchari Biswas, Ramakrishna Chinthala.

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