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Research Article

# Exploring seasonal behavior, metal exposure, and reproductive dynamics in captive vultures

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## Abstract

Vultures are vital scavengers that help maintain ecosystem balance, making their conservation crucial. In South Asia, particularly since the 1990s, vulture populations have declined drastically due to the veterinary use of diclofenac, with severe drops by the early 2000s. To aid recovery, the Punjab Wildlife Department, in collaboration with WWF, established the Changa Manga Vulture Conservatory in Punjab, Pakistan, focusing on the conservation of critically endangered White-rumped Vultures (*Gyps bengalensis*). Captive vultures were studied using non-invasive sampling of meat, water, feces, and feathers to assess heavy metal exposure. The samples were washed, labeled, digested using aqua regia, and analyzed using a PerkinElmer AAnalyst 200 Atomic Absorption Spectrometer. Twelve metals were quantified, categorized as heavy metals (Pb, Cd, Cr, Al, As) and essential metals (Cu, Mg, Mn, Fe, K, Na, Zn). Seasonal variations were observed, with Al, As, and Pb showing higher concentrations in winter. Behavioral observations revealed fear and alarm responses when humans approached closely, likely due to confinement and limited social interaction. Seasonal behavioral shifts were noted; winter prompted breeding behaviors like nesting and mating, alongside increased aggression due to territoriality, while summer saw reduced feeding and more walking activity. These behavioral patterns suggest captivity may not fully support vultures' natural ecological and social needs. Reproductive success was recorded, with the number of vultures rising from 2 to 36, indicating some success in rehabilitation. However, the study highlights the need for further research into environmental factors such as ambient air and additional contamination sources. Emphasis should also be placed on enhancing captive conditions to support vulture well-being and ensure long-term population sustainability.

**Keywords:** Atomic Absorption Spectrometer, Breeding, Behavior, Conservatory

## Introduction

Vultures are famous scavengers and are essential to ecosystems because they recycle nutrients back into the environment and eat dead animals. They help to control the spread of disease. They have vanished from southeast Asia since 1990 (Yasmeen et al., 2021). The decline was very high and by the 2000s most of the region's vulture populations had decreased by almost 95% and diclofenac was the important reason for its decrease (Galligan et al., 2021). Heavy metals are those with an atomic number greater than 20 and a specific gravity greater than 5 $g/cm^3$ and are considered major environmental contaminants because of their toxicity and persistence (Amengor, 2024; Piwowarska et al., 2024). The soil and water contamination are increasing due to industrialization and increased human activity, which is affecting ecosystems and wildlife (Weldeslassie et al., 2018; Sharma et al., 2023; Kolawole and Iyiola, 2023). Due to their high susceptibility to contaminants and status in the food chain, birds serve as valuable bioindicators of environmental health (Egwumah et al., 2017). Environmental discharge is a common consequence of human activities, including agricultural and industrial processes (Chowdhary et al., 2020). These harmful compounds can poison vultures and cause other serious health problems (Plaza et al., 2019; Mishra et al., 2019).

Although certain species, such as metallophyte plants and heavy metal-tolerant insects, exhibit relative resilience to the harmful effects of these metals, which vary depending on the species and environmental factors (Gall et al., 2015). Heavy metals can impede reproductive functions, damage immune systems, and eventually threaten the survival of species in birds, particularly vultures (Carneiro et al., 2015). Although these threats are being addressed through conservation initiatives, heavy metals' pervasiveness remains a serious ecological problem on a global scale (Yasmeen and Asif, 2022).

Heavy metals like Lead (Pb), cadmium (Cd), chromium (Cr), aluminum (Al), and arsenic (As) are examples of serious environmental pollutants that interfere with essential biological functions and pose serious health risks. They are persistent and could bioaccumulate in ecosystems. Particularly dangerous are Pb and Cd, where Pb can damage the nervous system, cause organ failure, and increase mortality (Pain et al., 2009; García-Fernández et al., 2018; Jafari et al., 2021; Zaynab et al., 2022), and Cd is connected to cancer and renal damage (Hernández-Cruz et al., 2022). While aluminum alters the metabolism of calcium and can cause long-term health problems, chromium has the potential to be poisonous and carcinogenic. Arsenic is extremely harmful and can damage several organ systems. Due to impaired immune system and reproduction, the buildup of these metals in vultures, one of the top predators, contributes to population decreases (Crnić et al., 2022; Yamaç et al., 2019; Pain et al., 2008; Sharma and Agrawal, 2020).

Essential metals like Copper (Cu), magnesium (Mg), manganese (Mn), iron (Fe), potassium (K), sodium (Na), and zinc (Zn) are necessary for cellular metabolism, enzyme function, and general health in both humans and wildlife. Mg maintains muscle and nerve function, while Cu helps with iron metabolism and nervous system function. Zinc is necessary for DNA synthesis and immune system function, iron is necessary for oxygen transport, and manganese is needed for bone formation and metabolism. Sodium and potassium control cellular homeostasis and are essential for nerve transmission and muscle contraction. On the other hand, overindulgence may result in harmful effects, including Mn-related neurotoxicity or zinc-induced copper shortage. Metal imbalances in birds can have an impact on their survival, growth, and reproduction (Jankowski et al., 2019; Kirchgessner et al., 2012; Zduńczyk and Jankowski, 2014).

A few studies emphasized the importance of monitoring heavy metal levels in birds to assess environmental pollution. Moreover, the non-invasive methods like examining blood, feathers, and feces provide insight regarding bioaccumulation of these metals in various species (Berglund et al., 2011; Yasmeen et al., 2018). The inability of heavy metals to biodegrade, their enduring presence in soils, and their potential to pose long-term risks to human populations and wildlife alike continue to be formidable obstacles (Khalef et al., 2022). Birds exposed to heavy metals over an extended period may experience oxidative stress, which can impair cellular processes and cause a range of health problems (Koivula and Eeva, 2010; Espín et al., 2014). It is well known that the vultures have vanished from the natural habitat of Pakistan and are now only available in captivity. Currently, the wildlife department of Pakistan is working for their conservation A conservatory is being built in Changa Manga. In research, there is very little data reported concerning Cd, Cr, Al, and As compared to lead. The present study was designed to fill the gaps and to examine behavior and assess bioaccumulative levels of certain essential and heavy metals in captive vultures in winter and summer seasons.

## Material and methods

This study was conducted in the Gyps Vulture Conservation Center, Changa Manga Forest Park, Punjab, Pakistan (Fig. 1). This center was built in collaboration with the World Wildlife Fund

(WWF) for the conservation of critically endangered white-backed vulture (*Gyps bengalensis*). The conservatory is built in an artificial forest that covers almost 12,000 acres to minimize disturbance to vultures and is in the Kasur District of Punjab. The permission was taken from wildlife management staff.

**Figure 1.** A vulture's nest in the Gyps Vulture Conservation Center

**Methodology and Collection of Samples**

A non-invasive sampling technique was used for the present study, and samples of fecal, meat, water, and feathers were randomly collected. A total of 36 vultures of different age groups, such as juveniles, subadults, and adults, were included that are part of the captive population and present at the vulture rehabilitation and conservation center at the time of sampling. Before studying, ethical approval for animal research and sample collection was taken by the ethical committee of Lahore Garrison University, Lahore. Samples of fecal material, fresh meat, feathers (barbules of feather B, rachis of feather R), and water were collected in summer (May to July) and winter seasons (December to March) in triplicate. The meat was taken from the conservatory-grown animals like donkeys, rabbits, and goats. The behavior of vultures was observed directly as well as monitored using already installed cameras (Fig. 2). Sample digestion for heavy metal analysis was done in the laboratory of Lahore Garrison University, DHA phase VI, Lahore, and the solution of digested samples was sent to Lahore University of Management Sciences, LUMS.

**Figure 2.** Monitoring of vultures via installed cameras

## Chemical Digestion of Samples

For chemical digestion, fecal and food samples were used as collected, while for the tail and wing feathers, they were first washed with acetone, dried, and further divided into two types of rachis and barbules of feathers. The fecal matter was ground in a mortar and sieved. The samples were then oven-heated at 200°C till the samples changed to ash. 0.5 grams of each sample was added to a flask with three ml of nitric acid ($HNO_3$) and one ml of perchloric acid ($HClO_4$). The flasks were heated until the mixtures changed color from pale yellow to brown and then became a clear solution. The samples were mixed with 20 ml of double distilled water, filtered and poured into screw capped test tubes. Two drops of hydrogen peroxide ($H_2O_2$) were added. A blank solution was also prepared with 1.5 ml of nitric acid ($HNO_3$) and 0.5 ml of perchloric acid ($HClO_4$), and 20 ml of double-distilled water (Yasmeen and Asif, 2022).

## PerkinElmer Analyst 200 Atomic Absorption Spectrometer

The prepared samples were analyzed for 13 heavy metals: Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb), Mercury (Hg), Aluminum (Al), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Zinc (Zn) using PerkinElmer AAnalyst Series atomic absorption spectrometer, (Analyst 200). The Analyst Series instrument was turned on and allowed to warm up, ensuring that the lamp was stabilized. For each element, the appropriate hollow cathode lamp (HCL) was selected, which emits light at the element's characteristic wavelength. Calibration curves were established using standard solutions of known concentrations. The absorbance of each sample was measured by exposing it to the characteristic wavelength of the corresponding element. Data analysis was performed using the calibration curves, and the concentrations of each element in the samples were recorded.

### Monitoring of seasonal behavior in captive vultures

The seasonal behavior of captive vultures was monitored by direct and indirect observations.

### Statistical Data Analysis

Data was entered and analyzed by using SPSS 25.0/ all the qualitative variables were presented by frequency and percentage, and quantitative by Mean and SD. Comparison among groups was conducted by applying the ANOVA test and a p-value <0.05 was considered significant.

## Results

The present study was designed to see seasonal change in behavior and concentration of metals in various samples of captive vultures.

### Concentration of Heavy metals in summer samples

The concentration of Heavy metals (Al, As, Cd, Cr, and Pb) in various samples, such as water, meat that was donkey meat, wings (wing R and B), and feces, was noticed in the summer season. It was seen that higher levels of Al were found in feces, followed by meat and water samples. There was more deposition in wing barbules compared to wing rachis. The trend was the same for As and Cd, but for the Cr highest levels were recorded for meat, followed by feces and water, with more bioaccumulation in wing R rather than wing B. However, a higher concentration of lead was recorded in water samples than in meat samples (Table 1). Data was also analyzed statistically by one-way ANOVA, and highly significant results were found at p-value 0.00 in samples and metals of the summer season.

**Table 1:** Concentration of Heavy metals in summer samples in µg/g

| Summer Samples | Water | Meat | Wing R | Wing B | Feces |
|---|---|---|---|---|---|
| **AI** | 88.44±2.06 | 139.08±12.78 | 133.05±0.64 | 412.83±1.31 | 584.93±7.16 |
| **As** | 0.24±0.31 | 1.32±1.78 | 0.58±0.08 | 0.74±0.08 | 3.10±0.15 |
| **Cd** | 0.11±0.014 | 0.44±0.367 | 0.18±0.05 | 0.46±0.05 | 0.55±0.035 |

| | | | | | |
|---|---|---|---|---|---|
| **Cr** | 4.37±0.22 | 15.22±14.42 | 6.92±0.14 | 5.87±0.21 | 12.78±0.33 |
| **Pb** | 14.64±17.35 | 17.59±7.42 | 16.5±0.42 | 26.45±0.63 | 17.54±0.74 |

### Concentration of Heavy metals in winter samples

The concentration of heavy metals (Al, As, Cd, Cr, and Pb) in various samples, such as water, meat that was donkey meat, wings (wing R and B), and feces, was noticed in the winter season. It was seen that higher levels of Al in feces were followed by meat and water. There was more deposition in wing barbules compared to wing rachis. The trend was the same for the As and Cd. For Cr, meat had with higher concentration, followed by feces and water, but a higher deposition was noticed in wing rachis than wing barbules. However, a higher concentration of lead was recorded in water than in meat was recorded while feces had with higher concentration of lead (Table 2). Data was also analyzed by one-way ANOVA, and statistically highly significant results were found at p-value 0.00 in samples and metals of the winter season.

**Table 2.** Concentration of Heavy metals in winter samples in µg/g

| Winter Samples | Water | Meat | Wing R | Wing B | Feces |
|---|---|---|---|---|---|
| **AI** | 85.44±2.36 | 150.08±0.05 | 132.01±0.7 | 416.68±0.71 | 685.86±121.6 |
| **As** | 0.36±0.07 | 2.19±1.41 | 0.57±0.11 | 0.54±0.01 | 4.09±0.14 |
| **Cd** | 0.10±0.007 | 0.58±0.46 | 0.15±0.04 | 0.45±0.04 | 0.91±0.08 |
| **Cr** | 4.34±0.04 | 15.22±11.59 | 6.28±0.69 | 5.15±0.05 | 14.34±0.47 |
| **Pb** | 125.2±35.28 | 16.16±2.8 | 18.5±0.71 | 28.55±0.78 | 16.55±0.7 |

### Concentration of Essential metals in summer samples

The concentration of essential metals (Cu, Fe, K, Mg, Mn, Na, and Zn) in various samples, such as water, meat that was donkey meat, wings (wing R and B), and feces, was noticed in the summer season. It was seen that higher levels of all essential metals in feces were followed by meat and water samples. There was more deposition in wing barbules compared to wing rachis (Table 3). Data was also analyzed statistically by one-way ANOVA, and highly significant results were found at p-value 0.00 in samples and metals of the summer season.

**Table 3.** Concentration of Essential metals in summer samples in µg/g

| Summer Samples | Water | Meat | Wing R | Wing B | Feces |
|---|---|---|---|---|---|
| **Cu** | 0.41±0.03 | 2.55±0.32 | 2.02±0.08 | 2.21±0.31 | 10.7±0.52 |

| | | | | | |
|---|---|---|---|---|---|
| **Fe** | 198.49±123.6 | 469.42±29.20 | 378.85±2.92 | 939.55±14.18 | 5725.65±92.28 |
| **K** | 125.87±27.87 | 1391.51±284.27 | 112.02±2.04 | 169.3±1.87 | 2685.18±77.71 |
| **Mg** | 943.8±734.36 | 1523.8±15.06 | 1412.76±21.28 | 1483.9±22.75 | 1519.79±44.97 |
| **Mn** | 15.43±0.54 | 19.03±1.83 | 19.81±0.27 | 47.76±0.79 | 136.75±1.48 |
| **Na** | 382.39±125.09 | 506.87±198.99 | 241.79±1.39 | 242.27±11.38 | 969.85±42.65 |
| **Zn** | 305.01±265.17 | 544.43±39.15 | 468.64±2.09 | 540.88±41.75 | 703.75±2.76 |

**Concentration of Essential metals in winter samples**

The concentration of essential metals (Cu, Fe, K, Mg, Mn, Na, and Zn) in various samples, such as water, meat that was donkey meat, wings (wing R and B), and feces, was noticed in the summer season. It was seen that higher levels of all essential metals in feces were followed by meat and water samples, except Cu, where the highest concentrations were found in meat as compared to fecal samples. There was more deposition in wing barbules compared to wing rachis (Table 4). Data was also analyzed statistically by one-way ANOVA, and highly significant results were found at p-value 0.00 in samples and metals of the winter season.

**Table 4.** Concentration of Essential metals in winter samples in µg/g

| Winter Samples | Water | Meat | Wing R | Wing B | Feces |
|---|---|---|---|---|---|
| **Cu** | 0.47±0.06 | 16.56±3.55 | 1.77±0.14 | 2.045±0.08 | 11.69±0.74 |
| **Fe** | 284.29±1.82 | 470.07±42.40 | 352.94±3.05 | 936.24±1.08 | 7288.4±561.30 |
| **K** | 125.64±6.41 | 1325.55±105.42 | 112.205±1.27 | 161.405±0.71 | 2689.74±227.00 |
| **Mg** | 1083.29±259.88 | 1507.79±10.86 | 779.905±892.09 | 1431.405±0.98 | 1763.44±2.79 |
| **Mn** | 14.97±1.32 | 18.68±3.55 | 25.87±0.33 | 48.75±0.81 | 139.705±11.02 |
| **Na** | 335.685±77.05 | 539.36±139.71 | 239.21±0.69 | 232.7±2.12 | 944.38±78.08 |
| **Zn** | 349.01±183.14 | 588.27±17.39 | 450.025±0.18 | 499.135±0.33 | 697.9±3.11 |

**Monitoring of Seasonal Behavior in Captive Vultures**

The seasonal behavior in captive vultures was monitored during the study period, and distinct patterns were recorded for summer and winter seasons. Nest-building activity was absent in summer, and vultures relied on existing winter nests. While in the winter season nest nest-building activity was recorded as high, which showed vultures preferred this season for nesting. Similarly, mating calls were not observed during summer, although mating behavior was witnessed on rainy days. However, mating was recorded in winter, which was characterized by calls governed by males. This showed winter was the favorite season for vultures for mating.

Aggression levels were higher in winter, attributed to the breeding season and the presence of eggs and nests. Vultures tend to sleep more in winter, particularly with their offspring. Parental care during the winter season appeared more prominent in females were more cautious and more caring to the offspring as compared to the males. While in the summer season vultures were less attentive, with females seemingly preferring more care for their offspring. Feeding behavior also decreased in summer compared to winter, and aggression levels were notably lower. While walking was more prominent in summer and egg laying typically occurred between November and March, with increased nest-building activity and parental dominance observed. Overall, the captive vultures exhibited distinct behavioral shifts between the two seasons, reflecting adaptations to varying environmental and reproductive conditions.

### Reproductive Dynamics in Captive Vultures

The vulture conservation project, commenced in 2005 (https://www.hawk-conservancy.org/wp-content/uploads/2023/04/Chaudhry-Murn_HB3-2022_Vultures.pdf), began with only eight birds, indicating a cautious start. Despite efforts, the production rate remained slow. However, over time, the dynamics evolved. In the year 2023, there were promising signs of progress as one egg was infertile out of four, while three others showed signs of fertility. Notably, the incubation process was primarily done naturally due to a failed experiment with machine incubation. With an average egg-laying age of 7 to 8 years, the total bird count reached 36, comprising seven pairs, totaling 14 individuals. Among these pairs, five were designated breeding pairs, while two were considered non-breeding pairs due to overage. Plans are underway to release these non-breeding older pairs into the environment to check their stability and versatility in the environment alternative to the native species, although a comprehensive strategy and further evaluation is required to assess their potential impact. It's noteworthy that out of the 36 birds, 13 had reached adulthood but had not yet commenced egg-laying activities. Additionally, there were nine individuals categorized as babies, signaling the potential for future pairing. Overall, the project reflected a journey of growth and adaptation, navigating challenges while steadily working towards its conservation goals.

## Discussion

The present study was designed to see seasonal changes in behavior and concentration of metals in various samples of captive vultures (*Gyps bengalensis*) examined at the Gyps Vulture

Conservation Center, located in the Changa Manga Forest and Wildlife Park in Punjab. The vultures were fed with meat that was grown in the conservatory, and other animals such as donkeys, goats, and rabbits were also housed in the same area. These animals were sacrificed daily and provided as food for the vultures. A total of 36 vultures were housed in captivity and were given approximately 1 kg of meat each day. The vulture's population declined in southeast Asia due to diclofenac and heavy metals and described by Paital et al. (2015) that in India and its adjacent nations, vulture carcasses have been found to contain traces of heavy metals, such as diclofenac and its derivatives, which may have contributed to vulture mortality through biomagnification.

Mikula et al. (2021) reported smelting, coal-fired power plants, and garbage disposal are the main sources of heavy metal contamination, releasing lead, mercury, and arsenic into the environment. In our research different heavy metals (Al, As, Cd, Cr, and Pb) have been studied in summer as well as winter season and a significant amount recorded in feathers. A study by Pikula et al. (2013) emphasized the serious consequences of lead poisoning as organ damage, anemia, and unsuccessful reproduction particularly in captive Cinereous and Egyptian Vultures due to soil contamination from lead-based paint. In our findings heavy metals were noticed in meat and water samples and a study by Espín et al. (2013) from Spain on Griffon vultures were reported and low levels of lead (Pb), cadmium (Cd), and mercury (Hg) recorded that significantly impact oxidative stress and antioxidant enzymes, the elevated Pb levels were due to contaminated game meat. Another study by Carneiro et al. (2015) on Griffon vultures in Portugal and Catalonia were found to have high lead (Pb) levels that might be due to local sources of contamination. Similarly, higher heavy metals particularly of Pb were recorded in water samples of our study and might be contaminated from surroundings. In another research study from South Korea, the tissues of dead Cinereous vultures had high amounts of lead, suggesting that lead poisoning was a primary cause of death. They most likely are exposed to lead while migrating or from their nesting and resting grounds (Nam and Lee, 2009). In one of study from Ludhiana reported high levels of arsenic (As), cadmium (Cd), and lead (Pb) in feces that were also recorded in current study (Kler et al., 2014). The higher levels of metals in excrement might be due to environmental pollution, eating contaminated food or water, and the bioaccumulation of metals in the tissues of the birds. According to Bassi et al. (2021) an increased lead levels were found in 44% of bird scavengers in south-central Europe, and 26% of them displayed clinical poisoning. primarily from ingesting lead from game shot. A study by (Rajamani and Subramanian, 2015) studied metal concentrations in various organs of White-backed Vultures to assess their role in population decline. High lead levels were recorded in some individuals' liver and kidney that suggest toxic effects, although most metal levels were within normal ranges. García-Fernández et al., (2005) depicted that vultures are susceptible to exposure to and buildup of environmental pollutants like pesticides and heavy metals as they are found at the top of the food chain. Nighat et al. (2013) also stated vultures fed on the carcasses of animals left in open fields, leading to higher levels of heavy metals in their bodies compared to other birds of prey. Yasmeen and Laiba, (2022) Pb, Cr, Cd reported higher level of metals in feces compared to present study. In the present study higher levels of Al, As, Cd, Pb, and Cr in captive vultures might be due to environmental contaminants such as lead-based paints, contaminated food, or exposure to contaminated water. It is essential to maintain clean surroundings and closely watch food sources to reduce these risks. López-Berenguer et al. (2021) reported comparable lead levels while Cr, and Cd levels in their study were lower compared to our findings and the change in these metals is due to immediate environment exposure.

The concentration of essential metals (Cu, Fe, K, Mg, Mn, Na, and Zn) in various samples such as water, meat, wings and feces were noticed in summer as well as winter season. It was seen that higher levels of all essential metals in feces followed by meat and water samples. While there was more deposition in wing barbules compared to wing rachis. López-Berenguer et al. (2021) also reported high levels of Cu, and Zn in vulture’s feathers and elevated concentrations were recorded in areas with greater mining activity that showed activities in surrounding environment effects living organisms. Pavlović et al. (2017) agreed that variation in seasons may result on bioaccumulation of metals such as Boron, Zinc, Strontium and Copper even in plants. Kara et al. (2014) also agreed about deposition of Fe, Zn, Mn, Pb, Cu, and Cr in winter and summer season from the surrounding environment. Similarly, it was reported in another study that the levels of heavy metals (Cd, Zn, Ni, Pb, Cu, and As) in raptor feathers varied greatly between seasons and geographical areas of Punjab like higher metal levels were recorded for the Central Punjab. Moreover, seasonal changes suggested that pollution levels were higher at different periods of the year. Another study by Ozaki et al. (2023) also depicted levels of lead, cadmium, and arsenic in common buzzards increased in late winter and decreased in late summer. while Cu levels exhibited the opposite pattern. Study also suggests the seasonal variations in these metals may be related to human activities like lead shot use and food. One more study by Kim and Oh, (2016) compared cinereous vultures in Korea to other prey birds, showed greater liver concentrations of iron (Fe), lead (Pb), and cadmium (Cd). Pb levels were elevated in these vultures, probably because of ingesting Pb shot.

The general behavior of vultures was monitored by direct and indirect methods. The direct method includes standing outside the cages while for indirect methods behavior was observed with already installed cameras. The mating behavior of the vultures was observed from November to March, during which time no intruders were allowed near the conservation cage area to avoid disrupting the mating process. Only one egg was laid by the vultures annually in captivity, and they reached maturity at the age of 4 to 6 years. It was noted that the captive vultures did not build nests in summer but in winters they built nests on treetops and exhibited aggressive behavior. They also showed fear and would become alarmed if humans approached within approximately 10 ft of them. If humans were detected, the vultures would fly to the treetops and emit alert calls to other group members. Aggression levels were higher in winter, attributed to the breeding season and the presence of eggs and nests and this was also reported in another study (Margalida and Bertran, 2005). According to a study males were more active compared to the females it was controversial to our study where females were active and nest building wis a main task before egg laying Margalida and Bertean, (2000). Margalida and Bertran, (2000) reported male bearded vultures were more involved in nest construction and defense before to laying and both sexes contribute equally to parenting duties while in our finding females were more active in nest building and parenting care like (Sharma and Sharma, 2011). As the chick matures, both parents gradually reduce their presence at the nest, sharing the tasks of incubation and chick feeding equally. A study by Morris and Slocum (2019) explains the observation of black vultures (*Coragyps atratus*) developing a habit of feather self-plucking in captivity underscored the detrimental impact of confinement on these birds. Feather plucking was a behavior commonly associated with stress, boredom, and environmental dissatisfaction in captive animals. In the case of black vultures, the confinement and restricted environment of captivity likely contributed to the manifestation of this behavior. Feather plucking could have serious consequences for the health and well-being of vultures. The act of plucking feathers could lead to skin irritation, inflammation, and even open wounds, increasing the risk of infection. The loss of feathers could compromise the vulture's ability to regulate body temperature and maintain proper insulation, making them more vulnerable to environmental stressors and highlighted the importance of providing enriched and stimulating environments for these birds in captivity. Another investigation by Dorey et al. (2009) represented a novel application of functional analysis in evaluating self-injurious behavior in birds. The research had indicated that employing differential reinforcement techniques can effectively mitigate problematic behaviors in non-human animals, especially in black vultures. Rabenold (1987) discussed and highlighted the role of human attention in maintaining self-injurious behavior in captive animals, contrasting with non-captive species. It showed that automatic reinforcement may have existed for both; social factors were absent in captivity. Black vultures' complex social structures in the wild underscored the potential influence of social reinforcement on behavior.

Chaudhary (2019) observed that Indian vultures that were released into their natural environment raised concerns about their ability to adapt and reintegrate into the wild. This confusion was attributed to disrupted social structures and unfamiliar surroundings. Limited social interactions in captivity could have contributed to difficulties in recognizing and interacting with other vultures, leading to potential conflicts within the group. The findings of Margalida et al. (2020) indicated that a captive environment was not comparable to the natural habitat of animals, often resulting in adverse health and behavioral effects. Bearded Vultures in the Spanish Pyrenees are mostly fed bone pieces, with calcium and phosphorus being the most common minerals found in their feces. The research verifies that bones are completely broken down, and although fecal studies shed light on certain aspects of diet, in-person observations of prey provide more precise nutritional data (Margalida et al., 2020). The increase in vulture's number from 8 to 35 showed success story of this rehabilitation center (https://www.wwfpak.org/our_work_/wildlife_2/vultures/saving_vultures_in_pakistan/)

## Conclusion

There was no seasonal variation in heavy and essential metal concentrations in captive vultures, despite significant metal presence in meat and water samples from animals raised in the conservatory. However, vultures exhibited distinct seasonal behaviors. From November to March, they displayed breeding activity, built nests in treetops, and showed increased aggression. They also reacted with fear when humans approached within 10 feet, likely due to confinement and limited social interaction. These findings suggest that captive environments may not fully meet vultures' ecological and social needs. Further research is needed to explore alternative conservation strategies focused on their well-being and sustainability.

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