DIFFERENCES IN BIOACCUMULATION OF HEAVY METALS AND METALLOIDS BETWEEN BARBS AND CALAMUS OF TAIL FEATHERS OF WHITE BACKED VULTURES (*GYPS AFRICANUS*)

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Research highlights:

- HMs and As were assessed in barb and calamus of tail feathers of *Gyps africanus*
- Lead and Cadmium were detected as the dominant metals in tail feathers
- Hg and As was not detected in any samples of barb and calamus
- Metals in barb were comparatively higher than in calamus of *Gyps africanus*
- Barb and calamus of tail feathers are validated as promising biomonitoring tools

ABSTRACT: Current study was designed to validate the use of tail feathers as non-invasive biomonitoring tool and to compare its barb and calamus parts for bioaccumulation of heavy metals viz. Cd, Cr, Pb, Cu, Hg and As in white backed vulture (*Gyps africanus*). A total of 8 tail feathers samples collected from eight birds were analyzed. All the studied heavy metals except Hg and As were detected in tail feathers of *G. Africanus*. We corroborated the use of tail feathers as non-invasive biomonitoring tool for all the heavy metals except Hg and As. In general, metals in feathers followed the trend as Pb>Cd>Cr>Cu. Concentrations of trace metals were higher in barbs than calamus reflecting possible external deposition. Comparison of heavy metals revealed non-significant ($P > 0.05$) differences between barbs and calamus parts. We concluded that barbs of feathers are promising biomonitoring tool for metals contamination.

Key words: birds, biomonitoring, concentration, differences, environment.

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INTRODUCTION

The elements that have high density (5g/cubic centimeter) and are toxic to living organisms, even in very low concentrations are termed as heavy metals (HMs here onward). These are in particularly lead (Pb), chromium (Cr), copper (Cu), cadmium (Cd), arsenic (As) and mercury (Hg). Heavy metals are natural constituents of earth's crust but indiscriminate anthropogenic activities have influenced their biochemical and geochemical cycles (Singh et al., 2011). Due to these off balances in natural cycles the ecosystems gets hugely disturbed and if a certain keystone specie gets choke down by a certain disease or any reagent like heavy metals, the whole ecosystem crumbles and all the other species present in that ecosystem also suffer these off balances (Ray et all, 2008).

Heavy metals being metals are reactive because of high electropositive values but their unique characteristic is relatively high electronegative values too as compared to other metals (Mande et all, 1990). This peculiar behavior of heavy metals makes them extremely grimmer, even in very low concentrations because they destroy the structures if the other element is not conducive in bond formation. And that is what exactly happens when heavy metals combine with living organisms. They form tumors and unreversed diseases. When a particular heavy metals enters in a food chain, due to its altitudinous reactivity it affects all from producers to tertiary consumers and if the detrimental effects somehow find their ways to a keystone specie, the whole ecosystem collapses.

As in the case of every pollution the heavy metals also have two main sources into the environment. I.e. natural and anthropogenic sources. The two sources release the heavy metals in air, surface or ground waters. These pollutions intricate themselves into the very internal parts of the environment i.e. food chain, where the drastic effects multiply synergistically. The natural sources of heavy metals form two groups; the first group includes rocks: magmatic, sedimentary and metamorphosis rocks are chief and principal sources of heavy metals into the environment. Apart from these the soil formation, oxides and hydroxides also have heavy metals in combined or bonded forms. The second group includes sources of heavy metals in surface and ground water. In these bodies the heavy metals travel from the mineral sources to near water bodies and then from there propagate to far places. In air and atmosphere, heavy metals travel naturally in the form of aerosols and particulates from mineral dusts, sea salts, fugitive particulate emissions, volcanic aerosols and forest fires (Bradl, 2005). In this way heavy metals can haunt an ecosystem naturally too if these produced by nature do not have proper mitigations.

The anthropogenic sources of heavy metals are also diverse and even more formidable than the natural ones. We have two groups here too. The first group includes Agricultural sources: from the agricultural activities our notorious elements find their way to environment through pesticides, phosphoric fertilizers (which also contain zinc and cadmium), bio solids and sewage effluents. According to Bradl, (2005) application of land for waste water is in wide practice in industrialized countries from last 50 to 100 years. The second group includes all industrial sources which comprises of mining, coal and petroleum combustion, solid waste disposal, and indoor or urban environments (mega cities usually refer to big populations. Big populations means big energy consumptions; that means more hazardous emissions in the environment). The anthropogenic sources are main apple of discord if remain unchecked and unbalanced without any barrier or standard. But in the 21st century the emissions of heavy metals are getting out of hands. The developed countries claimed to have addressed this issue, but these countries have made developing and third world countries their dumping grounds. So the problem of uncontrolled generation still remains out of hunt.

Heavy metals do occur naturally but increased amounts of them due to anthropogenic activities have seriously compromised the environment′s ability to foster life and has rendered its intrinsic values (Masindi &Muedi, 2018). The health of animals, plants and humans is under the threat due to bioaccumulation and bio magnification (the gradual accumulation of heavy metals in the food chains and multiple individuals from different species in an ecosystem) of non-degradable form of heavy metals. The remediation of these bioaccumulation is not sustainable at large scales, either too much capital consuming with very little effect or generating another harmful waste while clearing one. So in this way these heavy metals are in particular very toxic to environment. For the biota in aerial environment the presence of heavy metals are specifically very dangerous (Abbasi et all, 2015).

The birds also considered as bio indicators in the environment because they are very sensitive and most prone to get influenced by any change in the environment. In addition to that heavy metals present in the form of ultra-fine particles that can enter in the blood stream or till the end of organs and organ systems, these metals enter in the feathers of the birds and remain there for long intervals of times causing the whole or partial choke down of the individual (Manjula, et all, 2015). And according to Manjula, et all, (2015) the presence levels of certain heavy metals such as Cr, Cu and other found in almost equal quantities in rural and urban parts of the India. This shows us that how deep intoxication of heavy metals have happened in the different ecosystems regardless of the urban and rural regions.

Feathers can be a very efficient source of non-invasive biomonitoring as first we do not have to handle bird for the feathers and they can very easily be collected from the nests and from the fields over they fly, and secondly the deposition of toxic substances by the pumping activity of blood remains there for particular time period as an archive, that could be extremely injurious for the bird (Garcia et all 2013). These depositions of heavy metals can be both internal and external as when a bird fly through the heaps of heavy metals and fumes they lodge on the body of the bird. Furthermore, in the developmental stages of birds the feathers are connected with bloodstream via an artery that transports methyl Hg to form the creatinine structure of feathers but after fully evolved the levels of mercury remains same, until the exposure of bird to external mercury and other heavy metals like cadmium, lead chromium (Martinez et all, 2012). The presence of heavy metals and toxic substances also vary in different parts of the feather, the barbs may have different quantities whereas calamus of more in direct contact with blood stream also differ in values.

MATERIALS AND METHODS

Study Area: The research was conducted in the area of changa manga forest situated in province Punjab and district Kasur around 74 km south west from Lahore, the busiest city of Punjab. The forest is situated between latitude 31o 1′ to 31o 7′ north longitude 73o 56′ to 74o 4′ east latitude. The changa manga forest is present from five kilometers from the Multan road which is always very busy with traffic. The colossal sundar industrial state is also within the range of 50 kilometers (43.1 to be precise). Apart from that agricultural run-off is also a problem in the surroundings of the forest, as the main source of people to make both ends meet around the area is agriculture.

Study design:

Sample collection: The white back vultures(*Gyps africanus*) were kept under captivity there for their conservation purposes. As they are critically endangered species. The sample feathers were collected around or from inside of the cages as tool of non-invasive bio monitoring tool. Those samples then brought to the laboratory to carry out the digestion and further research.

Sample digestion: The feathers were measured in height first of all. After measurement they were washed with acetone and de ionized water in the lab. From the feathers, samples of 5 grams calamus (quill) and 5 grams of barbs were isolated for the digestion and identification of presence of heavy metals in the feathers. For digestion after washing the samples they were dried for one hour at 103 degree in the oven. Then the samples were digested in 3:1 mixture of nitric acid and hydrogen peroxide. For five grams sample 10 grams of nitric acid was used against 30 grams of hydrogen peroxide. The samples in this mixture were placed on hot plate for half an hour and then diluted with 25 grams of distil water and after that filtered with Whatman filter paper into the sample bottles. After that samples went for further analysis of Atomic absorption spectroscopy and induced couple plasma mass spectrometry.

Statistical analysis: After the filtration Atomic absorption spectroscopy was performed on the digested samples to diagnose the presence of four heavy metals. I.e. chromium, lead, cadmium and copper. For the detection of mercury and arsenic inductively couple plasma mass spectrometry was performed. The data obtained after the analysis was used to draw results. The data analysis tools present in the Atomic absorption spectrometer and Induced couple plasma mass spectrometer obtained the primary data for the results the research. In addition to that simple descriptive statistical analysis were made to further clarify the results.

RESULTS

Metals profiles in feathers: The heavy metals vary differently in numeric respect in different parts of the feathers. The detection frequency for four metals (Cr, Cd, Cu, and Pb) was cent percent, while for mercury and arsenic detection frequency was zero. In calamus the amount of lead is way more than the every other heavy metal, the cadmium was the second highest found metal in the calamus. Whereas the amounts of copper and chromium were fairly lower than those of cadmium and lead. In calamus the levels of arsenic and mercury remained undetected. The mean values were also went according to the trend, lead had the highest mean value, i.e. 0.206 while cadmium being the second with 0.149. However the mean values of copper and chromium were lower i.e. 0.057 And 0.061 respectively.

Table 1: Metals Mean ± S.D concentrations (Minimum-maximum) between Calamus and barbs of tail feathers of white backed vulture (*Gyps Africanus*). Test for significance (t-test) is shown in the bottom to depict comparison.

Part of feather	Cu	Pb	Cr	Cd
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
	Min –Max	Min -Max	Min -Max	Min -Max
Calamus	0.057 ± 0.020 0.036 - 0.083	0.206 ± 0.049 0.136 - 0.244	0.061 ± 0.012 0.05 - 0.078	0.149 ± 0.016 0.135 - 0.171
Barb	0.080 ± 0.027 0.046 - 0.111	0.419 ± 0.204 0.271 - 0.708	0.148 ± 0.053 0.11 - 0.223	0.179 ± 0.026 0.143 - 0.203
Test for significance t (*P*)	1.02 (0.36)	2.07 (0.22)	5.11 (0.08)	1.98 (0.23)

Table 2: Correlation matrix between the mean metal concentrations in barb and calamus of tail feathers of White backed vulture (*Gyps africanus*).

		Cu	Pb	Cr	Cd
Cu	R	1			
	P				
Pb	R	0.636	1		
	P	0.174			
Cr	R	0.338	0.201	1	
	P	0.513	0.702		
Cd	R	-0.172	-0.152	0.686	1
	P	0.744	0.774	0.132	

Mean values of the lead was highest with 0.419 and in one bird it was shockingly high with 0.708. Cadmium had the mean value of 0.179. Copper had the 0.080 and chromium was 0.148 respectively. Mercury and arsenic found undetected in the barbs too.

The detection frequency of Cd, Cr, Pb and Cu was 100 percent in barbs too and the other way around about mercury and arsenic. Also the trends of barbs were no different but the amounts of metals present inside the barbs were way more as it was found in the calamus. That's the storage in the form of archive we have discussed above in the literature. In barbs too, the values of lead was the maximum along with the cadmium levels being second best. But in the barbs the values of chromium were much eminent as compared to the calamus. The quantities of copper were also on the higher side when the comparisons were made with calamus.

T test between variables tells us the authenticity that whether the difference between different values is significant or not. A value is said to be significant if the difference between mean values is less than 0.05. A statistical significance is defined as the chances of having a divergence from the null hypothesis or a more extreme one, in a sample. Anova test for finding significance has shown the levels of significance in the values of different metals in the barbs and calamus of tail feathers of white back vultures. All the values appeared to be non-significant with Cu, Pb, Cr, and Cd having values 0.369, 0.224, 0.087 and 0.232 respectively for significance. So T test too implies that the metals present inside the barbs and calamus having irregular quantities and their presence does not depend on each other.

DISCUSSIONS

The general difficulties that are associated with the biomonitoring of birds for the presence and estimation of heavy metals were reduced in the study of heavy metals in tail feathers of white back vultures(*Gyps africanus*), because all the analysis were done in the same lab and with same apparatus and the uniformness was well maintained in the preparatory methods too. So the consistency followed in all the procedure has led us to some repercussions to discuss.

Lead was the most abundant heavy metal present inside our samples of tail feathers. The presence of this much quantity in white back vultures(*Gyps africanus*). Have more than one reason. The urbanization from rural to developed areas is a very burning problem in cities all around the world (Roux &Marra, 2007). The lead in the urban lands is significantly more in urban lands from the expected natural levels as compared to the rural lands. The primary anthropogenic source of lead in the urban environment all over the world is leaded gasoline. The combustion of this fuel and the ever increasing burden of automobiles on the roads of cities is major contributor of soil lead in the urban environment, even though lead based paint is also a contributor of lead into the soil and environment. As birds are bio indicators of the ecosystem so they are present on the first line of defense in the immune forces of an ecosystem. Another reason of poisoning of lead among hunter birds is eating of meat of the animals injured or killed by the lead based bullets (Fernandez et al, 2011). The site of our study have exposure to high levels of auto mobile combustion, in house lead based paints and hunting with lead bullets, so that’s the reason of high levels of lead in the feathers of the birds.

The levels of cadmium in birds depends upon degree of exposure to contaminated soil. The cadmium levels specifically increase inside the body of a bird from the contaminated environment it lives during the breeding season and feeding habits also brought the curse of cadmium (Bianchi, et al, 2008). Birds directly contact the soil from the habitat they live in and they also ingest the soil while eating. The soil cadmium increase by the usage of fertilizers containing cadmium and sewage sludge on farm lands drastically increase the levels of soil contaminations of cadmium (Järup&Åkesson, 2009).

Chromium is a non-essential element and accumulations of it cause complications in the reproductive systems of birds (Aziz, et al, 2021). The unceasing amounts of chromium in avian species has been recorded worldwide in the areas where leather industry is dominant. However apart from particular local areas chromium also increases in birds by contacting the birds that have migrated from the other areas where the levels of this toxic metal were high. The presence of chromium in birds can be from other sources than tanneries. In water it comes from the weathering of chromium containing rocks, the dumping of ferrochromium, slag and chromium plating baths (Oliveira, 2012). These sources increase the toxicity of chromium in environment multiple times. Birds in the area of our study also face this problem and have acquired the levels of chromium in the barbs and calamus of tail feathers.

Copper is an essential elements and found in trace elements during developmental stages in mammals and birds (Singhet all, 2012). The use of pesticides and mining activities are responsible for sinister amounts in environment. The values of copper in the barb and calamus of tail feathers of white backed vultures were relatively lower than the other metals. The area of the study is far from any sort of mining activity and the use of pesticides has decreased to a great degree since the usage of evolved seeds for agriculture. These evolved seeds have greater immunity so the minimum pests attack on the crop and the need of using a pesticide has decreased respectively (Parween,et all, 2016). The areas close to industrial activity are still susceptible to copper toxicology.

Conclusion: In a nutshell, we conclude that the heavy metals (Cu, Cd, Pb, Hg, Cr and As) are serious environmental concern. In the tail feathers of white backed vultures, lead was the most abundant in quantity in both barb and calamus. Cd was the second most copious after lead, whereas Cr and Cu were the 3rd and 4th respectively in manner of abundance. There were no evidence recorded for the presence of Hg and As in barb and calamus. The presence of above metals in barbs were significantly higher as compared to calamus. However the difference between the values of all the metals was not recorded significant according to T test. And the values were random. The correlation among the different metals were found in balance between a perfect correlation and no correlation at all. These elevated levels of heavy metals in barb and calamus of tail feathers show the high potentials of external contamination. The absence of Hg and As also shows the low contaminations and exposure through ingestion as these two accumulate mostly in this way.

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