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FECES OF CAPTIVE WILD MAMMAL USE AS BIO-INDICATOR OF HEAVY METAL POLLUTION IN URBAN AIR

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Abstract : Feces of captive wild mammals were collected from Udaipur zoological garden, Rajasthan to establish the level of heavy metals in atmosphere. Feces were treated with concentrated nitric acid and perchloric acid before analysis using atomic absorption spectrophotometer (AAS). The mean concentration value were found in range of lead (Pb) 3.9 to 16.70, cadmium (Cd) 0.43 to 4.43, Chromium (Cr) 0.55 to 6.19, Copper (Cu) 8.41 to 41.0, and Zinc (Zn) 10.1 to 40.15 µg/g (ppm). Analysis of feed and water along with the soil in cages which is receiving particulate air pollutants indicates that air pollution is the primary cause due to high density of traffic in the area.

Key words: Feces, Metals, Zoo mammals, Bio- indicator, Urban air.

I. INTRODUCTION

Monitoring trace metals in street dust has provided a tool for estimating the degree of contamination, source and habitat of residential commercial and industrial areas. Industrial street dust and motor vehicle emissions are sources of airborne particulates in urban environment [1]. The particles emitted by motor vehicles carry or contain heavy metals that may be toxic when present in excess of natural background levels [2]. The toxic properties of this airborne particulate may be due to the biochemical activity of metals attached to them [3]. The ingredient present in domestic airborne aerosol plays a significant role in toxicological effects. Being sufficiently small and insoluble, these would get adequate time to penetrate the deepest area of lungs triggering asthma attacks and aggravate suffering [3].

Zoological gardens (zoos) are institutions or facilities in which animals are confined within enclosures, displayed to the public, and in which they may also be bred. The history of modern zoological gardens, however, started some 200 years ago with the creation of the first public zoological garden. Since that time, large numbers of zoological gardens have been established in all parts of the world [4]. Globally, zoological gardens are known to offer great opportunities for entertainment and education, and to contribute to wildlife conservation and promote scientific research, especially for environmentalists and conservationists, as the rate of extinction of wild life increases.

Most of the zoos which were once located on the outskirts of the cities and towns are now surrounded by human activities like vehicular traffic and industries. Some of the famous zoos like municipal corporation zoo at Ahmadabad and forest departmental at Ahmadabad have vehicular traffic too close to premises. All these activities result in heavy metal pollution, which may be adversely affect the health and wellbeing of the wild animals housed in such protected areas. Udaipur, the last of the capitals of Mewar, was founded by Maharaja Udaisingh in 1867. It is popular as lake city. Udaipur is known to be an tourist destination. Udaipur zoo is located in public garden known as Gulab Bagh. Udaipur zoo is located in the centre of city surrounded by urban localities by motorable roads on which vehicles are frequently plying.

Death have been reported in captive wild animals including monkeys, bears, raccoons, armadillos etc. due to ingestion of lead containing paint.[5],[6]. Similar situation was also reported in domestic animals like dogs, cats, goats, cattle etc.[7]. Mammals near urban areas with dense vehicular traffic and also near metal mines and smelters had the highest burdens of lead [6].

Various studies have been reported metal concentrations in wild mammals living in highly contaminated area near smelters[8], chlor-alkali plant[9],[10], verges of heavily-used highways [11] and mines or mine waste sites[12],[13]. Several methods were employed to assess and draw a concentration profile of a variety of pollutants that might reach the wildlife habitats and wildlife itself. In fact the human race in its selfish design has used wildlife species as biological indicators to study the ambient concentration of the toxicants in his own ecosystem, both urban and industrial. However, mammals, which are much closer to human beings, are rarely used. Rats, captured from either side of the highways indicated that concentration of the lead in the body was directly proportional to the distance from the highway [14].

Guano was first used as bio-indicator in Bat for pesticidal pollution as well as mercury exposure [15],[16],[17] and analysis cadmium in the feces of humans [18]. concentration of cadmium, lead, zinc, copper were reported in the feces of deer killed near smelters to check the degree of metals pollution [19].

A study was done in wild herbivores housed in various protected areas of Rajasthan, India clearly suggests that herbivore feces can be used as a bio-indicator of heavy metals exposure[20]. Similarly, study was also done in mammalian fauna of Keoladeo National Park, Bharatpur[21], Sariska Tiger Reserve, Alwar[22], Desert National Park, Jaisalmer and Gajner Wildlife sanctuary, Bikaner of Western Rajasthan[23], Jodhpur zoological garden[24]and Kota zoological garden[25]. Scat samples of the mammals, vegetation, and soil samples clearly indicate the extent to which the mammalian fauna is exposed to metal contamination.

The method of killing or sacrificing animal is not ethically sound. It is a purely invasive method which is increasing biological poverty on the earth. So there is an urgent need to develop a non-invasive method for monitoring heavy metal exposure. In our study we use feces / scat / fecal matter as bio-indicator of heavy metal contamination in wild or captive zoo mammals.

I. MATERIALS AND METHODS

A. *Sampling area*

Feces, feed, soil and water samples were collected from cages where wild mammals housed in Udaipur Zoological garden, Rajasthan.

B. *Sampling Procedure*

Fresh scat samples of mammals housed in the animal section of Udaipur zoo, India, were collected from the cages with the help of zoo staff. Samples were brought to the laboratory and freeze dried. Scat samples were collected from the cages of following mammalian species; Black buck (*Antilope cervicapra*), Chinkara (*Gazella gazelle*), Chital (*Axis axis*), Nilgai (*Boselaphus tragocamelus*), Sambar (*Cervus unicolor*), Rhesus monkey (*Macaca mulatta*), Indian porcupine (*Hystrix indica*), Rabbit (*Oryctolagus cuniculus*), Himalayan bear (*Melurus ursinus*), Wild boar (*Sus scrofa*), Fox (*Vulpes vulpues*), Asiatic lion (*Panthera leo*), Tiger (*Panthera tigris*), Panther (*Panthera pardus*). To ascertain the source of contamination water and food samples of this zoo were also collected. Another, suspected source of contamination was suspended particulate matter settling on the floor of cages, hence soil samples were also taken from cages of animals. Scat and soil samples were stored in the plastic zip lock bags and water samples in the sterilized plastic containers.

C. *Sample treatment*

For analysis of sample 0.5 gm of dry scat / vegetation / feed / soil were weighed and taken in the hard Borosil glass tube. Concentrated nitric acid and perchloric acid were added to each sample in 4:1 ratio. Sample was kept in water bath for 5 to 6 hours or until it was digested completely and became clear. When the sample was clear 3 to 4 drops of H_2O_2 (30%) were added to neutralize and to dissolve the fat. After cooling each sample was diluted upto 10 ml with deionized water and transferred to sterilized Borosil glass vial and stored at room temperature prior to analysis.

Water samples were transferred into beakers, cleaned with double distilled and acidified distilled water, and concentrated keeping on a hot plate in a flame hood adding 12 to 15 ml of analytical grade HNO_3. The heating was continued till such time the sample became colorless and clean. However, samples were never allowed to dry completely. By and large, nitric acid alone was adequate for complete digestion of water samples. $HClO_4$ was added only to those samples which had high organic matter which were always treated in advance (pre-treated) with nitric acid before adding perchloric acid. If necessary, more HNO_3 was added and volume brought down to the lowest quantity (10 to 25 ml) before precipitation occurred. After completing the digestion, beakers were allowed to cool. Samples were diluted upto 10 ml with double distilled water.

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D. Sample analysis

Sample analysis Quantitative determination of the trace metals was performed by an GBC Advanta ver. 1.31 Atomic Absorption Spectrophotometer (AAS) at 217 nm for lead, 228.9 nm for cadmium, 324.7 nm for copper, 213.9 nm for zinc and 357.9 nm for chromium. Results are presented in µg/g (ppm) dry weight and µg/ml (ppm) wet weight.

E. Statistical analysis

$$\text{Metal concentration} = \frac{\text{Dilution factor}}{\text{Weight of sample}}$$

Where,
Dilution factor=10
Dry weight of the sample= 0.5 gms

The statistical calculations were based on Ipsen and Feigel's[26] method. The values are expressed as mean ± standard deviation (S.D.) as well as in standard error (S.E.).

II. RESULTS AND DISCUSSION

Concentration of lead, cadmium, chromium, copper and zinc in scat / fecal matter was analysed for every mammalian species captivated in a similar environment of zoo. These results show a trend of variation in metal content according to the feeding habits as well as activity level of mammals. The mammals were categorized in three major groups i.e. herbivores that feed on green leaves (vegetation), vegetables, green grains, fruits, cereals, pulses etc., omnivores which feed on both vegetation and meat or fish and carnivores type which are fed meat and fish. Metals concentrations indicate gross exposure.

The concentration of lead analyzed in fecal matter of captive zoo wild mammals was in the range of 16.70±1.05 (*Panthera tigris*) to 3.9±0.30 (*Panthera pardus*) ppm d/w. Cadmium was in range between 4.43±0.77 (*Cervus unicolor*) to 0.38±0.12 (*Panthera tigris*) ppm d/w. Chromium was in rage of 6.19±0.81 (*Macaca mulatta*) to 0.55± 0.37(*Vulpes vulpues*) ppm d/w. Copper was in range between 41.0±5.04 (*Hystrix indica*) to 8.41±0.18 (*Panthera leo*). Whereas zinc was found in range of 40.15±1.11 (*Panthera pardus*) to 10.1±1.45 (*Oryctolagus cuniculus*) ppm d/w (Table I).

The background levels of lead, cadmium, chromium, copper and zinc in food were analysed. The feed of every mammalian species was analyzed and it was found that lead was present in each sample of food which was provided to zoo mammals (Table II). The concentration of lead was found in the range of 6.12 to 11.0 ppm d/w. Cadmium was found in range of 1.17 to 2.12 ppm d/w. The concentration of chromium was found in the range of 1.99 to 9.92 ppm d/w. Copper was analysed in the range of 12.15 to 21.9 ppm d/w. The concentration of zinc in feed samples was observed in the range of 10.19 to 20.15 ppm d/w.

The background level of lead, cadmium, chromium, copper and zinc in soil and water from herbivore as well as carnivore cages were also analysed. The concentration of lead in soil was found to be 2.91±0.74 ppm d/w. Water was found to have trace amount of lead contents 0.38±0.58. Cadmium concentration in soil and water significantly lower i.e. 0.03±0.01 ppm d/w and 0.27±0.21 ppm w/w. Chromium concentration in soil and water were found to be significantly high i.e. 19.25±1.19 and 10.1±1.3 ppm w/w. Copper concentration of soil was also high i.e.19.78±0.16 and in water it was 3.84±0.51 ppm d/w. In case of soil and water, zinc content was 17.18±1.81 ppm d/w and Not detected respectively.

Lead, cadmium, chromium, copper and zinc concentration were found in considerable amount in the biological samples (fecal matter/ feed) and non-biological (soil/water) samples collected from Udaipur zoo. Concentration of metals in particularly in fecal matter samples from zoo is much higher than the wild animals like white tailed deer feeding near smelter[27]. Metal pollution in soils is derived mostly from atmospheric fallout, coal fly ash and bottom ash, urban refuse, animal wastes, and agricultural and food wastes[28]. Study of Udaipur zoo shows that a part of exposure of mammals is through food while the metals in water were in traces. Metal concentration in feces normally equals that in food[29]. Obviously the additional exposure was through plausible route of inhalation. The load of lead in fecal matter almost exceeded what is present in the food material.

Udaipur zoo apparently is polluted one for a traffic density much higher close to the zoo. However, the food is comparatively less contaminated but higher concentration metals in soil is indicative of heavy deposition of particulate matter. Wild mammals housed in zoo have no choice but to inhale the automobile exhaust, being caged, all 24 hours.

Soils receive potentially toxic elements from both natural and wide range of anthropogenic sources, including the weathering of primary minerals, mining, fossil fuel combustion, the metallurgical, electronic, and chemical industries, and waste disposal and automobile exhaust. Earlier studies have quantified deposition of metals in the vicinity of the highway or traffic dense area, either by measurement by dry depositions fluxes at various distances from road, or by calculating soil and vegetation concentrations and assuming that the soil acts as long term store, hence effectively integrating the deposition[30],[31]. Lead concentrations as high as 6835, 1180 and 682 ppm dry weight have been reported in soil, vegetation and invertebrates, respectively[31],[32].

Major sources of metals are irrigation water (when contaminated by sewage and industrial effluent), battery production, metal products, metal smelting, cable coating industries, brick kilns, automobile emissions, re-suspended road dust and diesel generator sets[33],[34],[35],[36]. Other sources can include unsafe or excessive application of pesticides, fungicides and fertilizers, and can also include sewage sludge[37],[38],[39],[40].

Metals belong to the group of foreign materials that are excreted into bile and their ratio of concentration in bile verses plasma is greater than 1.0 and may be as high as 10 to 1000. Since liver is in a very advantageous position for removing toxic materials from blood after their absorption, it can prevent their distribution to other parts of the body. Furthermore, because the liver is the main site of biotransformation of toxic agents the metabolites may be excreted into bile[41]. Lead is absorbed in gastrointestinal tract by two steps process. It is first absorbed from lumen and then excreted into the intestinal fluid[42]. Upon oral ingestion about 5 to 10 % of lead is absorbed and usually less then 5% of what is absorbed is retained[43]. Thus about 99.5 % of total ingested lead is excreted through feces. Out of this 90% is coming out without being absorbed and 9.5% after being absorbed and metabolized leaving only 0.5% to be deposited in various body tissues.

Fecal matter analysis method’s distinct advantages over tissue analysis are that the exposure can be measured on daily basis, it does not involve killing or even disturbing the wild mammals, it represents the metal eliminated which has been incorporated due to gross exposure (inhalation, ingestion or dermal exposure) in a locality. Thus, it can be concluded that wild mammals housed in Udaipur zoo are exposed to metallic pollution (air and water). Our study has firmly established the value of fecal matter analysis as bioindicator of heavy metal contamination. Thus analysis of feces has advantage that it indicates gross exposure, does not involve disturbing and killing the animals and monitoring of exposure to contamination at 24 hours intervals. The study can be further extended to free-ranging wild animal which are exposed to contaminants that are emitted by vehicles plying on roads within the protected areas.

III. Conclusion

Our study holds promise to develop a non-invasive tool for assessing environmental heavy metal pollution as well a step ahead for wildlife conservation.

Acknowledgement

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Table I: Metal concentration in scat samples of wild mammals housed in Udaipur Zoological Garden, Rajasthan

S.N.	Species	N	Pb(ppm)	S.E.	Cd(ppm)		Cr(ppm)		Cu(ppm)		Zn(ppm)	
			Mean±S.D.		Mean±S.D.	S.E.	Mean±S.D.	S.E.	Mean±S.D.	S.E.	Mean±S.D.	S.E.
	Scat of mammal											
1	*Antilope cervicapra*	10	10.36±1.24	0.373	2.54±0.15	0.049	5.16±0.88	0.329	26.5±0.5	0.353	29.52±1.65	0.94
2	*Gazella gazelle*	10	8.68±0.56	0.179	2.33±0.08	0.026	3.54±0.56	0.395	17.0±0.06	0.042	17.05±0.91	0.171
3	*Axis axis*	14	8.96±0.32	0.101	1.81±0.513	0.162	1.55±0.45	0.318	25.2±0.2	0.141	25.10±0.88	0.35
4	*Boselaphus tragocamelus*	12	9.70±0.41	0.130	1.67±0.061	0.019	0.94±0.68	0.480	12.27±0.07	0.049	18.82±1.33	1.08
5	*Cervus unicolor*	11	10.20±0.70	0.224	#4.43±0.77	0.244	1.13±0.29	0.110	16.73±0.39	0.275	22.19±1.25	1.09
6	*Macaca mulatta*	8	4.36±0.42	0.135	3.48±0.28	0.088	#6.19±0.81	0.279	10.12±0.81	0.142	31.34±1.12	0.56
7	*Oryctolagus cuniculus*	23	14.06±1.21	0.382	0.99±0.03	0.009	0.96±0.52	0.367	26.53±1.19	1.01	*10.1±1.45	0.831
8	*Hystrix indica*	12	9.64±1.01	0.320	1.14±0.74	0.236	2.12±0.02	0.014	#41.0±5.04	3.81	30.16±1.06	0.159
9	*Melurus ursinus*	11	9.74±1.11	0.351	1.97±0.14	0.046	1.34±0.58	0.410	35.87±2.91	1.69	20.19±1.31	0.88
10	*Sus scrofa*	9	4.21±0.94	0.343	1.37±0.26	0.084	3.44±0.98	0.407	11.13±2.81	1.98	15.10±0.34	01.51
11	*Vulpes vulpues*	20	7.08±0.60	0.190	0.43±0.05	0.017	*0.55±0.37	0.261	11.31±0.25	0.176	29.45±1.08	0.81
12	*Panthera leo*	10	10.66±1.85	0.586	2.35±0.20	0.063	2.13±1.15	0.813	*8.41±0.18	0.127	35.14±1.91	0.85
13	*Panthera tigris*	15	#16.70±1.05	0.332	*0.38±0.13	0.038	5.49±0.49	0.346	12.34±0.78	0.551	32.71±1.05	0.78
14	*Panthera pardus*	10	*3.90±0.30	0.094	1.81±0.36	0.115	5.03±0.11	0.134	8.61±0.97	0.685	#40.15±1.11	0.83

N= Number of samples

= Highest mean values µg/g (ppm)

* = Lowest mean values µg/g (ppm)

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Table II: Metal concentration in Feed, Soil and Water Samples from Udaipur Zoological Garden, Rajasthan

S.N.	Sources	N	Pb(ppm)		Cd(ppm)		Cr(ppm)		Cu(ppm)		Zn(ppm)	
I	**Food**		**Mean±S.D.**	**S.E.**	**Mean±S.D.**	**S.E.**	**Mean±S.D.**	**S.E.**	**Mean±S.D.**	**S.E.**	**Mean±S.D.**	**S.E.**
A	Meat	10	4.95±0.77	0.243	1.12±0.03	0.009	2.01±1.05	0.33	10.61±0.18	0.056	19.18±1.01	0.19
B	Fish	9	4.15±1.66	0.553	*1.11±0.04	0.013	3.33±1.1	0.36	10.17±0.03	0.01	*15.57±0.71	
C	Vegetation(Lucerne)	8	4.39±1.08	0.382	1. 47±0.47	0.166	*0.39±0.03	0.01	15.2±0.94	0.33	16.71±1.31	0.33
D	Vegetables	10	4.01±0.91	0.287	1.45±0.17	0.148	0.31±0.04	0.012	#19.28±1.71	0.541	#27.6±1.2	0.67
E	Fruits	6	2.7±1.21	0.495	#2.7±0.14	0.057	0.84±0.24	0.098	15.6±0.9	0.36	19.5±0.32	0.68
F	Cereals	9	6.05±0.42	0.14	2.2±1.5	0.5	1.88±0.36	0.12	9.04±1.02	0.34	21.4±1.7	0.24
G	Pulses	7	#7.6±0.34	0.128	1.16±0.06	0.022	#3.4±1.68	0.63	9.81±1.33	0.503	22.6±1.12	0.27
H	Sugar	10	*1.98±1.11	0.351	1.98±1.14	0.36	0.76±0.14	0.04	*5.14±0.71	0.224	16.6±1.28	0.40
II	Water	12	0.38±0.58	0.167	0.27±0.21	0.060	10.1±1.3	0.37	3.84±0.51	0.147	ND	-
III	Soil	15	2.91±0.74	0.191	0.03±0.01	0.002	19.25±1.19	0.30	19.78±0.16	0.040	13.72±0.35	0.093

N = Number of samples

ND = Not detectable

* = Lowest mean values µg/g (ppm)

= Highest mean values µg/g (ppm)