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Mammalian Feces as Bio-Indicator of Heavy Metal contamination in Bikaner Zoological Garden, Rajasthan, India

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Abstract

Present study is an attempt to evaluate feces as bioindicator of heavy metal contamination in captive zoo mammals. This is a non-invasive technique to study gross exposure of metal pollution. Various metal contents in mammals of Bikaner (India) zoo were in the range of 58.4±3.14 (Cervus unicolor) to 1.82±0.96 (Panthera. tigris) ppm d/w. Cadmium was in range between 2.46±0.08 (Axis axis) to 0.41±0.03 (Macaca mulatta) ppm d/w. Chromium was in rage of 91.68±2.28 (Oryctolagus cuniculus) to 1.36± 0.36(Macaca mulatta) ppm d/w. Copper was in range between 22.82±2.18 (Panthera pardus) to 6.15±0.45 (Boselaphus tragocamelus). Whereas zinc was found in range of 35.6±1.35 (Canis aureus) to 8.15±0.45 (Boselaphus tragocamelus) ppm d/w. 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.

Keywords: Bioindicator, feces, heavy metals, wild mammals, zoological garden.

Introduction

All organisms modify their environment, and humans are no exception. As the human population has grown and the power of technology has expanded, the scope and nature of this modification has changed drastically. Until recently, the term “human-dominated ecosystems” would have elicited images of agricultural fields, pastures, or urban landscapes; now it applies with greater or lesser force to all of Earth. Heavy metals are metallic chemical elements that have a relatively high density and are toxic or poisonous at low concentrations. Excessive concentrations in biological systems are detrimental;destabilize ecosystems because of their bioaccumulation in organisms, and toxic effects on biota and even death in most living organisms[1]. The bioaccumulation means an increase in the concentration of toxicant in biological organism over time, compared to the toxicant concentration in the environment. Biomonitors or indicators are any species that provides additional information about the health of an environment.

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[2]. 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. Kota is known to be an industrial area. Bikaner zoo is located in the centre of city surrounded by urban localities by motorable roads on which vehicles are frequently plying.

Rao Bikaji founded the exotic desert city of Bikaner in 1488. Bikaner zoo when established was located on outskirts, and is known as morden market. Public offices surround one of the sides. The tourist moving towards Junagargh fort move across the public park where the traffic density seems to be much higher.

Death have been reported in captive wild animals including monkeys, bears, raccoons, armadillos etc. due to ingestion of lead containing paint[3,4]. Similar situation was also reported in domestic animals like dogs, cats, goats, cattle etc[5]. 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[7], chlor-alkali plant[8,9], verges of heavily-used highways[10] and mines or mine waste sites[11,12]. 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[13].

Guano was first used as bio-indicator in Bat for pesticidal pollution as well as mercury exposure[14-16] and analysis cadmium in the feces of humans[17]. Concentration of cadmium, lead, zinc, copper were reported in the feces of deer killed near smelters to check the degree of metals pollution[18].

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[19]. Similarly, study was also done in mammalian fauna of Keoladeo National Park, Bharatpur[20], Sariska Tiger Reserve, Alwar[21], Desert National Park, Jaisalmer and Gajner Wildlife sanctuary, Bikaner of Western Rajasthan[22], Jodhpur zoological garden[23]and Kota zoological garden[24]. 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.

Methodology

Sampling Procedure: Fresh scat samples of mammals housed in the animal section of Kota 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 cases of following mammalian species; black buck (*Antilope cervicapra*), chinkara (*Gazella gazelle*), chital *(Axis axis),* nilgai *(Boselaphus tragocamelus),* sambar *(Cervus unicolor),* rhesus monkey *(Macaca mulatta),* bonnet monkey *(Macaca radiate),* Indian porcupine *(Hystrix indica),* rabbit *(Oryctolagus cuniculus),* sloth bear *(Melurus ursinus),* wild boar *(Sus scrofa),* hyena *(Hynea hyena),* jackal *(Canis aureus),* 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.

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.

Analytical determination: Entire metal analysis was done by using GBC Advanta ver. 1.31 Atomic Absorption Spectrophotometer 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.

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

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

Statistical analysis: The statistical calculations were based on Ipsen and Feigel's[25] method. The values are expressed as mean ± standard deviation (S.D.) as well as in standard error (S.E.).

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 58.4±3.14 (*Cervus unicolor*) to 1.82±0.96 (*Panthera. tigris)* ppm d/w. Cadmium was in range between 2.46±0.08 (*Axis axis*) to 0.41±0.03

(*Macaca mulatta*) ppm d/w. Chromium was in rage of 91.68±2.28 (*Oryctolagus cuniculus*) to 1.36± 0.36 (*Macaca mulatta*) ppm d/w. Copper was in range between 22.82±2.18 (*Panthera pardus*) to 6.15±0.45 (*Boselaphus tragocamelus*). Whereas zinc was found in range of 35.6±1.35 (*Canis aureus*) to 8.15±0.45 (*Boselaphus tragocamelus*) ppm d/w (table 1).

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 2). 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.

Table-1
Metal concentration in scat samples of wild mammals housed in Bikaner 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*	22	2.24 ± 1.19	0.378	1.09 ± 0.31	0.099	4.79 ± 1.69	1.19	8.83 ± 1.81	1.27	10.61 ± 1.98	0.30
2	*Gazella gazelle*	12	16.6 ± 0.38	0.174	#2.46 ± 0.08	0.027	7.47 ± 1.01	0.714	14.54 ± 0.58	0.117	20.15 ± 1.13	0.18
3	*Axis axis*	22	5.2 ± 1.32	0.420	1.0 ± 0.11	0.034	10.17 ± 0.17	0.12	10.09 ± 1.23	0.869	19.83 ± 0.95	0.53
4	*Boselaphus tragocamelus*	16	10.14 ± 0.87	0.277	1.72 ± 0.04	0.013	5.53 ± 1.43	1.01	*6.15 ± 0.45	0.318	*8.15 ± 0.45	0.81
5	*Cervus unicolor*	14	#58.4 ± 3.14	1.51	1.66 ± 0.36	0.011	13.53 ± 4.67	3.30	11.74 ± 1.01	0.981	10.98 ± 0.89	0.98
6	*Macaca mulatta*	9	5.2 ± 1.59	0.503	*0.41 ± 0.03	0.65	*1.36 ± 0.36	0.02	8.96 ± 0.64	0.452	17.15 ± 1.21	0.86
7	*Macaca radiate*	7	7.84 ± 1.94	1.06	0.74 ± 0.07	0.02	6.2 ± 1.26	0.305	16.66 ± 0.92	0.65	16.73 ± 1.25	0.89
8	*Hystrix indica*	12	3.09 ± 0.16	0.053	1.04 ± 0.21	0.06	18.53 ± 0.29	0.205	11.97 ± 0.71	0.528	17.70 ± 2.11	1.85
9	*Oryctolagus cuniculus*	8	12.62 ± 2.94	0.932	1.01 ± 0.06	0.02	#91.68 ± 2.28	1.91	15.98 ± 0.78	0.551	12.25 ± 1.01	0.72
10	*Melurus ursinus*	15	12.38 ± 4.95	1.568	0.63 ± 0.06	0.021	5.63 ± 1.75	1.23	8.58 ± 0.03	0.021	25.81 ± 0.32	0.28
11	*Sus scrofa*	10	17.6 ± 2.94	0.931	1.73 ± 0.186	0.059	63.83 ± 1.95	0.891	20.14 ± 0.75	0.325	15.10 ± 0.34	01.51
12	*Hynea hyena*	15	5.81 ± 2.41	0.761	0.97 ± 0.05	0.016	47.53 ± 1.53	0.915	15.66 ± 0.45	0.139	31.18 ± 1.09	0.31
13	*Canis aureus*	13	3.12 ± 0.73	0.231	0.62 ± 0.11	0.037	9.87 ± 1.87	0.971	21.66 ± 2.98	1.22	#35.6 ± 1.35	0.93
14	*Panthera leo*	16	2.6 ± 0.70	0.227	1.6 ± 0.047	0.014	17.8 ± 1.36	1.11	16.47 ± 0.98	0.742	29.81 ± 1.36	0.15
15	*Panthera tigris*	11	*1.82 ± 0.96	0.306	2.15 ± 0.13	0.041	39.47 ± 1.93	1.038	18.93 ± 1.09	0.731	28.23 ± 1.05	0.90
16	*Panthera pardus*	12	1.96 ± 1.42	0.450	1.73 ± 0.18	0.059	15.03 ± 0.19	0.134	#22.82 ± 2.18	1.54	35.16 ± 1.20	0.15

N = Number of samples, ND = Not detectable, * = Lowest mean values µg/g (ppm)

Table-2
Metal concentration in Feed, Soil and Water Samples from Bikaner 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	12	8.98 ± 1.03	0.297	1.26 ±0.07	0.02	4.02 ±0.28	0.08	16.05 ±1.09	0.315	16.22 ±0.66	0.19
C	Vegetation (Lucerne)	15	6.16 ±0.44	0.18	1.54 ±0.08	0.032	2.83 ±0.63	0.025	14.81 ±0.32	0.131	10.66 ±0.82	0.33
D	Vegetables	10	#11.0 ±1.62	0.57	#2.12 ±0.076	0.026	3.65 ±0.13	0.02	#21.9 ±0.10	0.035	*10.19 ±1.90	0.67
E	Fruits	9	*6.12 ±1.19	0.396	1.61 ±0.018	0.006	*1.99 ±0.17	0.056	*12.15 ±1.23	0.41	#20.15 ±2.05	0.68
F	Cereals	19	7.36 ±0.64	0. 171	1.32 ±0.11	0.029	#9.92 ±0.14	0.03	13.98 ±0.46	0.122	16.88 ±0.91	0.24
G	Pulses	11	9.69 ±1.52	0.481	*1.17 ±0.142	0.044	4.72 ±0.74	0.23	12.45 ±1.15	0.307	18.42 ±1.02	0.27
II	Water											
i	Herbivore Cage	7	ND	-	0.61 ±0.25	0.091	5.28 ±0.1	0.037	8.15±1.35	0.511	ND	-
ii	Carnivore Cage	5	ND	-	1.37 ±0.81	0.331	9.73 ±1.83	0.75	10.99 ±1.05	0.430	ND	-
III	Soil	30	8.36 ±1.90	0.508	1.06 ±0.093	0.024	7.63 ±1.69	0.451	10.02 ±0.08	0.021	13.72 ±0.35	0.09 3

N = Number of samples, ND = Not detectable, * = Lowest mean values µg/g (ppm), # = Highest mean values µg/g (ppm).

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 significantly high 8.36±1.90 ppm d/w. Water was found to have trace amount of lead contents i.e. not detectable. Cadmium concentration in soil and water significantly lower i.e. 1.06±0.093 ppm d/w and 0.61±0.25 (herbivore cages) to 1.37±0.81 (carnivores cages) ppm w/w. Chromium concentration in soil and water i.e. 7.63±1.69 and 5.28±0.17 (herbivore cages) 9.73±1.83 (carnivores cages) ppm w/w. Copper concentration of soil and water were found to be 10.02±0.08 ppm d/w and 8.15±1.35(herbivore cages) 10.99±1.05 (carnivores cages) ppm w/w. In case of soil and water zinc content was 13.72±0.35 ppm d/w and Not detected in herbivore as well as carnivore cages 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 Bikaner 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[26]. 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[27]. Study of Bikaner 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[28]. 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.

Bikaner 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 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[29,30]. Lead concentrations as high as 6835, 1180 and 682 ppm dry weight have been reported in soil, vegetation and invertebrates, respectively[31,30].

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[32-35]. Other sources can include unsafe or excessive application of pesticides, fungicides and fertilizers, and can also include sewage sludge[36-39].

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[40]. Lead is absorbed in gastrointestinal tract by two steps process. It is first absorbed from lumen and then excreted into the intestinal fluid[41]. Upon oral ingestion about 5 to 10 % of lead is absorbed and usually less then 5% of what is absorbed is retained[42]. 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 Bikaner 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 scat 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.

Conclusion

Our study indicate that scat can be use as better bio-indicator than other methods and it clearly establishes as a non-invasive tool for assessing metal exposure in wild or captive zoo mammals. It helps to conserve the wildlife.

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