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Exposure of Captive Wild Mammals in Kota Zoo India to Urban Air Pollution

KEYWORDS: Air pollution, Heavy metals, Bioindicator, Feces, Wild mammals.

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ABSTRACT *Study of environmental contamination in the wildlife, free-ranging or those caged in the zoos, is a challenging venture. This is primarily because of difficulty in obtaining samples, which can be at the most opportunistic, from these animals. Feces of wild animals were used as biological indicator of exposure in wild animals caged and exposed to the ambient air pollution of urban habitat. Various metal contents in mammals of Kota (India) zoo were in the range of 24.12±3.42 (Panthera leo) to 5.94±1.29 (Axis axis) ppm d/w in case of lead. Cadmium was in range between 14.36±1.6 (Panthera. tigris) to 1.09±0.52 (Panthera pardus) ppm d/w. Chromium was in rage of 45.47±1.98 (Melurus ursinus) to ND (Macaca radiate, Oryctolagus cuniculus, Panthera pardus) ppm d/w. Copper was in range between 32.5±0.90 (Sus scrofa) to 1.42±0.91 (Panthera leo) ppm d/w. Whereas zinc was found in range of 100.91±1.2 (Panthera leo) to 13.15±0.98 (Oryctolagus cuniculus) 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.*

Introduction

Toxic metals are natural components of the earth's crust found throughout the ecosphere in at least small (or "background") concentrations. These background concentrations are harmless to living organisms. Human activities, however, can cause concentrations of toxic metals to reach levels that pose hazards to living organisms. Some of these activities include burning of fossil fuels, metal refining, agriculture, mining operations, and wastewater discharge. For most of the toxic metals, the quantities of these substances mobilized by humans far outweigh the amounts that would naturally cycle through air, soil, and water of the earth [1]. .

Toxic metals are present in oil and coal, and metal-contaminated particles are released into the atmosphere by combustion of these fuels. Toxic metals are also released into the atmosphere by were emitted. A small fraction of these particles, however, becomes suspended in the upper atmosphere and is transported great distances from its source. As a consequence of this long-range transport, unnaturally high concentrations of toxic metals have been found in glaciers and lake sediments in the most remote regions of the Northern Hemisphere [2]. In urban areas industries and automobiles release large amounts of toxic metals into the air that eventually settle onto the ground.

The development of industry and motorization, as well as the continuing over-intensive use of various chemical compounds in agriculture leads to a constant increase of the levels of metals in the environment; being non biodegradable, they readily accumulate to toxic levels [3. The anthropogenic heavy metal contamination affects nowadays large areas worldwide [4] and excessive levels of metals could be introduced into the agricultural ecosystems through industrial waste or fertilizers [5]. Determining the concentrations of metals in the biota provides information concerning their movement through the environment, accumulation and potential toxicological effects [6]. Animals, especially mammals, are useful bioindicators for environmental monitoring in ecosystems with pollution loads [7].

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 [8]. 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. Kota zoo is located in the centre of city surrounded by urban localities by motorable roads on which vehicles are frequently plying.

Ingestion of Pb containing paint from bars and walls have been reported to be a significant cause of death among captive wild animals including monkeys, bears, raccoons, armadillos etc. [9],[10]. Similar situation was reported for domestic animals like dogs, cats, goats, cattle etc. [11]. The highest body burdens of Pb were reported in mammals near urban areas with dense vehicular traffic and also near metal mines and smelters [12].

Several studies have reported concentrations of metals in wild mammals living in highly contaminated area near smelters [13], chlor-alkali plant [14],[15], verges of heavily-used highways [16] and mines or mine waste sites [17],[18].

Various 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 the body concentration of the lead was directly proportional to the distance from the highway [19].

Bat was the first mammal used by analysis of its guano as bioindicator for pesticidal pollution as well as mercury exposure [20],[21],[22] and analysis of feces for Cd intake in humans [23]. Sileo et al.[24] recorded concentration of cadmium, lead, zinc, copper in the feces of deer killed near smelters to check the degree of metals pollution. David et al. [25] have found that fecal level of metals were four fold higher in urban than the rural rats.

A pilot study to monitor Pb contamination in wild herbivores from the protected areas of Rajasthan, India [26] suggests that exposure to heavy metals can be studied using herbivore dung as a bio-indicator. In the continuation of this, study was also done in mammalian fauna of Keoladeo National Park, Bharatpur [27], Sariska Tiger Reserve, Alwar [28], Desert National Park, Jaisalmer and Gajner Wildlife sanctuary, Bikaner of Western Rajasthan [29], Jodhpur zoological garden [30]. Scat samples of the mammals, vegetation, and soil samples clearly indicate the extent to which the mammalian fauna is exposed to metal contamination.

However, the method of sacrificing or killing of animal may appear more scientific, but is certainly ethically unsound. Given the concern for loss of animal lives for scientific investigation, and the increasing biological poverty of the planet earth, there is an urgent need for developing biological indicator which will not involve killing of animals. To overcome this problem it was proposed to use feces / scat / fecal matter as bio-indicators or as a biomarkers to study exposure to heavy metals.

Material and methods

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; chital (Axis axis), nilgai (Boselaphus tragocamelus), chinkara (Gazella gazella), sloth bear (Melurus ursinus), rabbit (Oryctolagus cuniculus), wild boar (Sus scrofa), Asiatic lion (Panthera leo persica), leopard (Panthera pardus), tiger (Panthera tigris), striped hyena (Hynea hynea), Guinea pig (Cavia porcellus), Bonnet monkey (Macaca radiata). 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 H2O2 (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 HNO3. 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. HClO4 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 HNO3 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.

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 24.12±3.42 (Panthera leo) to 5.94±1.29 (Axis axis) ppm d/w. Cadmium was in range between 14.36±1.6 (Panthera. tigris) to 1.09±0.52 (Panthera pardus) ppm d/w. Chromium was in rage of 45.47±1.98 (Melurus ursinus) to ND (Macaca radiate, Oryctolagus cuniculus, Panthera pardus) ppm d/w. Copper was in range between 32.5±0.90 (Sus scrofa) to 1.42±0.91 (Panthera leo). Whereas zinc was found in range of 100.91±1.2 (Panthera leo) to 13.15±0.98 (Oryctolagus cuniculus) ppm d/w (Table 1).

Table 1: Metal concentration in scat samples of wild mammals housed in Kota 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	Gazella gazelle	25	23.36±0.38	0.122	2.18±0.04	0.012	5.98±0.84	0.593	17.86±0.42	0.10	24.12±0.18	0.05
2	Axis axis	20	*5.94±1.29	0.408	2.29±0.11	0.035	8.11±0.99	0.70	21.70±1.01	0.777	23.11±1.59	0.38
3	Boselaphus tragocamelus	18	18.02±2.10	0.666	3.75±0.99	0.314	7.34±0.46	0.325	12.36±0.91	0.841	21.52±1.52	0.86
4	Macaca radiate	9	19.8±3.73	1.181	2.76±0.08	0.026	*ND	-	14.1±1.86	0.087	22.1±1.02	0.11
5	Oryctolagus cuniculus	10	15.18±4.13	1.308	2.09±0.13	0.043	*ND	-	5.57±0.07	0.026	*13.15±0.98	0.55
6	Melurus ursinus	15	14.16±0.50	0.159	10.55±1.41	1.01	#45.47±1.98	1.21	13.4±1.84	1.01	35.12±1.32	0.59
7	Sus scrofa	19	16.48±2.01	0.637	2.79±0.08	0.028	*ND	-	#32.5±0.90	0.636	38.05±1.99	0.53
8	Hynea hyena	14	10.06±0.58	0.185	11.39±2.4	0.76	0.33±0.15	0.021	16.09±0.81	0.279	31.62±1.52	0.161
9	Cavia porcellus	21	14.4±0.97	0.309	1.84±0.06	0.02	6.82±1.22	0.862	28.31±1.61	1.13	29.18±1.32	0.51
10	Panthera leo	20	#24.12±3.42	1.084	1.66±0.09	0.009	2.71±0.03	0.021	*1.42±0.91	0.017	#100.91±1.2	0.33
11	Panthera tigris	13	17.08±2.31	0.73	#14.36±1.6	0.532	0.03±0.01	0.009	14.83±0.23	0.015	89.91±1.98	0.52
12	Panthera pardus	10	15.12±1.85	0.585	*1.09±0.52	0.164	*ND	-	15.96±0.68	0.187	51.59±0.59	0.414

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

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 0 to 5.13 ppm d/w. Cadmium was found in range of 0.63 to 2.62 ppm d/w. The concentration of chromium was found in the range of 0.24 to 0.99 ppm d/w. Copper was analysed in the range of 6.41 to 15.7 ppm d/w. The concentration of zinc in feed samples was observed in the range of 18.5 to 76.1 ppm d/w.

Table 2: Metal concentration in Feed, Soil and Water Samples from Kota 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	3.92±0.08	0.023	*0.63±0.03	0.04	*0.24±0.11	0.039	*6.41±1.05	0.303	27.15±1.18	0.34
C	Vegetation(Lucerne)	15	4.41±0.19	0.047	1.13±0.05	0.01	0.28±0.09	0.023	#15.7±0.72	0.186	47.8±0.82	0.21
D	Vegetables	10	2.6±0.82	0.27	#2.62±0.97	0.01	#0.99±0.44	0.14	11.9±0.90	0.30	49.9±1.22	0.40
E	Fruits	9	1.49±0.7	0.22	1.98±0.18	0.32	0.78±0.11	0.03	11.3±0.62	0.083	#76.1±1.87	0.59
F	Cereals	19	3.18±0.99	0. 33	1.46±0.27	0.094	0.51±0.09	0.03	13.5±0.54	0.18	39.6±1.32	0.44
G	Pulses	11	#5.13±0.24	0.098	*0.63±0.05	0.256	0.29±0.10	0.251	10.83±0.15	0.06	45.81±1.98	0.81
H	Sugar	25	*ND	-	2.5±0.16	0	0.41±0.18	0.102	12.7±0.2	0.06	*18.5±1.64	0.51
II	Water	34	0.35±1.03	0.32	0.64±0.07	0.003	ND	0.11	3.35±1.03	0.32	ND	-
III	Soil	30	9.18±3.81	0.98	1.48±0.04	0.01	7.33±0.13	0.033	26.8±1.31	0.33	59.39±2.11	0.54

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 were also analysed. The concentration of lead in soil was found to be significantly high 9.18±3.81 ppm d/w. Water was found to have trace amount of lead contents i.e. 0.35±1.03 ppm w/w. Cadmium concentration in soil and water significantly lower i.e. 1.48±0.04 ppm d/w and 0.64±0.07 ppm w/w. Chromium concentration in soil and water i.e. 0 and 7.33±0.13 ppm w/w. Copper concentration of soil and water were found to be 26.8±1.31 ppm d/w and 3.35±1.03 ppm w/w. In case of soil and water zinc content was 59.39±2.11 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 Kota 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 [24]. Study of Kota zoo shows that a part of exposure of mammals is through food while the metals in water were in traces. Leonzio and Massi et al. [31] had shown that metal concentration in feces normally equals that in food. 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.

Kota zoo apparently is with most polluted one for a highway passing 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. National Ambient Air Quality Monitoring Programme [32] during had conducted air monitoring studies showing the presence of metals in the urban air of Kota city which appears to be the possible route of exposure and presence of metals indicates the gross exposure of wild mammals cages in zoos in immediate past 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 [33],[34]. Lead concentrations as high as 6835, 1180 and 682 ppm dry weight have been reported in soil, vegetation and invertebrates, respectively [35],[34].

Metal depositions are associated with a wide range of sources such as small scale industries (including battery production, metal products, metal smelting and cable coating industries) ; brick kilns ; vehicular emissions ; re-suspended road dust and diesel generator sets. These can all be important contributors to the contamination found in vegetables. In general, coal combustion is an important source, because Indian coal is of relatively poor quality and has high heavy metal contents. Additional potential sources of heavy metals in field locations in urban and peri-urban areas include irrigation water contaminated by sewage and industrial effluent leading to contaminated soils and vegetables. Other sources can include unsafe or excess application of (sometimes banned) pesticides, fungicides and fertilizers such as sewage sludge [36].

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 [37]. Lead is absorbed in gastrointestinal tract by two steps process. It is first absorbed from lumen and then excreted into the intestinal fluid [38]. Upon oral ingestion about 5 to 10 % of lead is absorbed and usually less then 5% of what is absorbed is retained [39]. 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 Kota 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. At least our study holds out a promise where scat can be used, since it does not involve either disturbing or killing of an animal, as useful bioindicator. 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.

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