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Mammalian Scat as a Bio-indicator of Heavy Metals Contamination in Western Rajasthan, India

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Abstract- Our aim to establish the metal (Pb, Cd, Cr, Cu and Zn) levels of wild mammalian scat samples deriving from sanctuaries of Western Rajasthan and compare the concentrations between Desert National Park, Jaisalmer and Gajner Wildlife sanctuary, Bikaner. Restrictions on the sampling because of Wildlife Protection Act (1972) prevents taking of samples of living tissues to analyse body burdens of contaminants that the wildlife may be carrying. According to our results the concentrations of metals (µg/g (ppm) dry weight) in fecal samples were in the range of 1.02 to 1.88 (Pb), 0.90 to 1.49 (Cd), 1.14 to 1.69 (Cr), 0 to 34.3 (Cu) and 0 to 25.53 (Zn) in DNP whereas in Gajner it was in range between 0.40 to 2.17 (Pb), 0.96 to 2.6 (Cd), 4.44 to 9.9 (Cr), 3.63 to 22.23 (Cu) and 4.61 to 10.15 (Zn). Background concentration in Soil, vegetation samples were also analysed . Detail data on contamination is presented in this paper.

Index Terms- Scat, Heavy metals, Bio indicator, Wild mammals, Western Rajasthan.

I. INTRODUCTION

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 (Adie and Osibanjo 2009). The anthropogenic heavy metal contamination affects nowadays large areas worldwide (Nicholson et al. 2003) and excessive levels of metals could be introduced into the agricultural ecosystems through industrial waste or fertilizers (Alloway 1990). Determining the concentrations of metals in the biota provides information concerning their movement through the environment, accumulation and potential toxicological effects (Torres and Johnson 2001). Animals, especially mammals, are useful bioindicators for environmental monitoring in ecosystems with pollution loads (Tataruch and Kierdorf 2004)

Several studies have reported concentrations of metals in wild mammals living in highly contaminated area near smelters (Beyer et al., 1985), chlor-alkali plant (Dustman et al., 1972; Wren, 1985), verges of heavily-used highways (Clark,1979) and mines or mine waste sites (Roberts et al., 1978; Andrew et al., 1984). A wide range of physiological and ecological effects of air pollutants in animals has been reported (Newman 1980). The effects ranged from physiological effects including death, to ecological effects such as behavioral changes. Environmental changes can be monitored biologically and non-biologically, directly in the field or using field samples in laboratory. Non-biological monitoring, since organisms generally integrate the effects of environmental contaminants over a period of time. However, using analytical chemistry in conjunction with appropriate biomarkers and bio-indicators can actually improve the environmental monitoring.

Wildlife sanctuaries, Desert National Park (DNP), Jaisalmer., and Gajner Wildlife Sanctuary, Bikaner., are situated in western Rajasthan. Desert National Park is an excellent example of the ecosystem of the Thar desert and its divers fauna. An area of 3162 sq kms and is situated 45 kms south-west from Jaisalmer. The major landform consists of craggy rocks and compact salt bottoms. The average rainfall is 100mm or less and the maximum temperature is 47 - 48 °C, minimum is 0 – 2 °C. In DNP, the vegetation is of xerophytic nature due to the dry, adverse climatic conditions. The chinkara, *Gazella gazelle*., Indian wolf, *Canis lupus*., desert cat, *Felis libyca*., desert fox, *Vulpes vulpues*., monitor lizard, *Varanus griseus*, and the rarest the great Indian bustard, *Choriotis nigriceps*., the spiny tailed lizard, *Uromastrix hardwickii*, more like phrehistoric animal, and desert hare, *Lepus nigricollis dayanus*, are the main animals. Gajner sanctuary is located at 27* 57 'N latitudes 73* 03 'E longitudes and 233 meter MSL altitude. It is situated about 25 kms south-west of Bikaner which covers an area of 38 sq kms and shows typical arid conditions; extreme high temperature, low annual rainfall, xerophytic vegetation and animals inhabiting the sanctuary are well adapted to desert conditions.

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. In one such study 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 (Way et al., 1982).

Bat was the first mammal used by analysis of its guano as bio-indicator for pesticidal pollution as well as mercury exposure (Reidinger, 1972; Petit and Altenbach, 1973; Clark et al., 1982) and analysis of feces for Cd intake in humans (Kjellstrom et al., 1978). Sileo et al (1985) recorded concentration of cadmium, lead, zinc, copper in the feces of deer killed near smelters to check the degree of metals pollution.

A pilot study to monitor Pb contamination in wild herbivores from the protected areas of Rajasthan, India (Gaumat and Bakre, 1998) 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 (Gaumat and Bakre, 2001) and Sariska Tiger Reserve, Alwar (Gupta and Bakre, 2012). 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.

I. Materials and Methods

In the field (Sanctuaries of western Rajasthan) scat sampling was totally opportunistic type. Fresh scat samples of wild mammals of reserves were collected with the help of forest staff from different sites. Samples were brought to the laboratory and kept in freeze for metal analysis. Scat samples of the following mammalian species were collected; Blackbuck., *Antilope cervicapra,* Nilgai., *Boselaphus tagocamelus,* Chinkara., *Gazella gazelle*, Wildboar., *Sus scrofa* from Gajner Sanctuary; Chinkara., *Gazella gazelle,* Blackbuck., *Antilope cervicapra,* Desert fox., *Vulpes vulpues* , desert hare., *Lepus nigricollis dayanus,* Desert cat., *Felis libyca* from DNP, Jaisalmer. To ascertain the source of contamination soil and vegetation(xerophytic) samples of these parks were also collected. Scat, vegetation and soil samples were stored in the plastic zip lock bags.

For analysis of sample 0.5 gm of dry scat / vegetation / 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.

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.

II. Results and Discussion

Wildlife sanctuaries studied in Western Rajasthan were Desert National Park (DNP),and Gajner sanctuary. The scat/fecal matter sample analysis shows the presence of lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu) and Zinc (Zn) in varying concentrations. In DNP, concentration of lead was observed in the range of 1.02 to 1.88 ppm d/w wheras it was 0.40 to 2.17 ppm d/w in Gajner sanctuary. Cadmium was in range of 0.90 to 1.49 ppm d/w in DNP , whereas it was 0.96 to 2.6 ppm d/w in Gajner sanctuary. Concentration of chromium was 1.14 to 1.69 ppm d/w in DNP, whereas it was 4.44 to 9.9ppm d/w in Gajner sanctuary. Concentration of copper was 0 to 34.3 ppm d/w in DNP and 3.63 to 22.23 ppm d/w in Gajner sanctuary. Zinc was in range of 0 to 25.53 ppm d/w in DNP whereas in Gajner sanctuary it was in range 4.61 to 10.15 ppm d/w in the fecal samples of wild mammals. Lead concentration was found in as follows in different mammals. In blackbuck, *Antilope cervicapra*, it was DNP (1.12±0.31 ppm d/w) > Gajner sanctuary (0.42±0.15 ppm d/w). In Chinkara., *Gazella gazelle*, the order was DNP(1.32±0.73 ppm d/w) > Gajner sanctuary (0.40±0.11 ppm d/w). In DNP, Concentration of lead in Desert hare, *Lepus nigricollis dayanus* it was 1.88±0.57 ppm d/w, in Desert fox., *Vulpes vulpues it was* 1.47±0.19 ppm d/w, in Desert cat., *Felis libyca* it was 1.02±0.91 ppm d/w. In Gajner sanctuary concentration of lead was in Nilgai., *Boselaphus tagocamelus,* it was 1.69±0.62 ppm d/w, in wild boar., *Sus scrofa*, it was 2.17±0.80 ppm d/w. (Table I, II)

The concentration of cadmium was found in as follows in different mammals. In blackbuck, *Antilope cervicapra*, it was Gajner sanctuary (2.12±0.21ppm d/w) > DNP (1.21±0.97 ppm d/w). In Chinkara., *Gazella gazelle*, the order was DNP(1.19±0.06 ppm d/w) > Gajner sanctuary (0.96±0.05 ppm d/w). In DNP, Concentration of lead in Desert hare, *Lepus nigricollis dayanus* it was 0.90±0.05 ppm d/w, in Desert fox., *Vulpes vulpues* it *was* 1.34±0.04 ppm d/w, in Desert cat., *Felis libyca* it was 1.49±0.91 ppm d/w. In Gajner sanctuary concentration of lead was in Nilgai., *Boselaphus tagocamelus,* it was 2.6±0.09 ppm d/w, in wild boar., *Sus scrofa*, it was 0.98±0.10 ppm d/w. (Table I, II)

The concentration of chromium was found in as follows in different mammals. In blackbuck, *Antilope cervicapra*, it was Gajner sanctuary (9.9±2.72 ppm d/w) > DNP (1.51±0.87 ppm d/w). In Chinkara., *Gazella gazelle*, the order was Gajner sanctuary (4.44±1.18 ppm d/w) DNP(1.15±0.09 ppm d/w). In DNP, Concentration of lead in Desert hare, *Lepus nigricollis dayanus* it was 1.14±0.04 ppm d/w, in Desert fox., *Vulpes vulpues* it *was* 1.43±0.01 ppm d/w, in Desert cat., *Felis libyca* it was 1.69±0.49 ppm d/w. In Gajner

sanctuary concentration of lead was in Nilgai., *Boselaphus tagocamelus*, it was 7.88±1.49 ppm d/w, in wild boar., *Sus scrofa*, it was 7.27±1.74 ppm d/w. (Table I, II)

The concentration of copper was found in as follows in different mammals. In blackbuck, *Antilope cervicapra*, it was Gajner sanctuary (6.36±2.01 ppm d/w) > DNP (*ND). In Chinkara., *Gazella gazelle*, the order was Gajner sanctuary (20.80±4.19 ppm d/w) DNP (*ND). In DNP, Concentration of lead in Desert hare, *Lepus nigricollis dayanus* it was 17.09±0.35 ppm d/w, in Desert fox., *Vulpes vulpues* it *was* 34.3±2.91 ppm d/w, in Desert cat., *Felis libyca* it was 18.24±1.87 ppm d/w. In Gajner sanctuary concentration of lead was in Nilgai., *Boselaphus tagocamelus*, it was 3.63±0.42 ppm d/w, in wild boar., *Sus scrofa*, it was 22.23±5.21 ppm d/w. (Table I, II)

The concentration of zinc was found in as follows in different mammals. In blackbuck, *Antilope cervicapra*, it was Gajner sanctuary (7.61±1.13 ppm d/w) > DNP (*ND). In Chinkara., *Gazella gazelle*, the order was Gajner sanctuary (6.81±1.08 ppm d/w) DNP(*ND). In DNP, Concentration of lead in Desert hare, *Lepus nigricollis dayanus* it was ND*, in Desert fox., *Vulpes vulpues* it *was* 25.53±1.35 ppm d/w, in Desert cat., *Felis libyca* it was 20.54±2.49 ppm d/w. In Gajner sanctuary concentration of lead was in Nilgai., *Boselaphus tagocamelus*, it was 10.15±1.56 ppm d/w, in wild boar., *Sus scrofa*, it was 4.61±1.98 ppm d/w. (Table I, II)

The analysis of soil and vegetations (Xerophytic) indicated that metals i.e. lead, cadmium, chromium, copper and zinc were present in background concentrations in both sanctuaries. (Table I, II)

Heavy metal concentrations were found in background amount in the biological samples collected from sanctuaries of western Rajasthan. Desert National Park, and Gajner sanctuary have little vehicular movement and there is no urban settlement nearby. This is reason that most of the biological samples of these sanctuaries are showing background concentrations.

Leonzio and Massi et.al. (1989) 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.

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 (Little and Wiffen 1977,1978**)**. Lead concentrations as high as 6835, 1180 and 682 ppm dry weight have been reported in soil, vegetation and invertebrates, respectively (Williamson and Evans 1972, Little and Wiffen 1978).

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 (Klaassen 1976**)**. Lead is absorbed in gastrointestinal tract by two steps process. It is first absorbed from lumen and then excreted into the intestinal fluid (Sobel et al. 1938). Upon oral ingestion about 5 to 10 % of lead is absorbed and usually less then 5% of what is absorbed is retained (Goyer 1986). 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.

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.

III. CONCLUSIONS

Our results shows that fecal matter can use as good bio-indicator for gross metal exposure and it provide a less expensive or better means of assessing long-term trends in pollution or other forms of environmental change. This method is completely non-invasive one to conserve the wildlife.

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Table I : Metals concentrations in Biological samples of Desert National Park (DNP), Jaisalmer, Rajasthan

S.N.	Species	N	Pb (ppm)		Cd(ppm)		Cr (ppm)		Cu (ppm)		Zn (ppm)	
			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.
	Scat of Wild Mammal											
1	*Gazella gazelle*	10	1.32±0.73	0.23	1.19±0.06	0.019	1.15±0.09	0.02	*ND	-	*ND	-
2	*Antilope cervicapra*		1.12±0.31	0.33	1.21±0.97	0.31	1.51±0.87	0.75	*ND	-	*ND	-
3	*Vulpes vulpues*	6	#1.47±0.19	0.06	#1.34±0.05	0.015	#1.43±0.01	0.003	#34.3±2.91	0.92	#25.53±1.35	0.42
4	*Lepus nigricollis dayanus*	8	*1.88.±0.57	0.20	*0.90±0.05	0.017	*1.14±0.04	0.014	17.09±0.35	0.12	*ND	-
5	*Felis libyca*		1.02±0.91	0.59	1.49±0.91	0.11	1.69±0.87	0.09	18.24±1.87		20.54±2.49	0.86
	Vegetation											
6	*Capparis deciduas*	10	*1.06±0.12	0.03	*ND	-	*1.23±0.05	0.015	*9.27±0.03	0.009	*ND	-
7	*Lasiurus scindicus*	12	#1.96±0.15	0.04	*ND	-	#1.51±0.05	0.014	#14.1±0.12	0.034	#6.6±1.12	0.32
8	Soil	19	1.58±0.27	0.18	*ND	-	1.91±0.03	0.006	4.09±0.11	0.025	7.92±1.08	0.24

N=Number of samples, ND= Not detectable, * =Lowest Mean values, # = Highest Mean values, Metal concentration in µg/g (ppm) dry weight.

S.N.	Species	N	Pb (ppm)		Cd(ppm)		Cr (ppm)		Cu (ppm)		Zn (ppm)	
			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.
	Scat of Wild Mammal											
1	*Antilope cervicapra*	20	0.42±0.15	0.03	2.12±0.21	0.05	#9.9±2.72	0.60	6.36±2.01	0.44	7.61±1.13	0.25
2	*Gazella gazelle*	31	*0.40±0.11	0.01	*0.96±0.05	0.008	*4.44±1.18	0.21	20.8±4.19	0.75	6.81±1.08	0.19
3	*Boselaphus tragocamelus*	35	1.69±0.62	0.14	#2.6±0.09	0.007	7.88±1.49	0.13	*3.63±0.42	0.04	#10.15±1.56	0.13
4	*Sus scrofa*	20	#2.17±0.80	0.17	0.98±0.10	0.023	7.24±1.43	0.32	#22.23±5.21	1.16	*4.61±1.98	0.44
	Vegetation											
5	Juliflora sp.	10	1.05±0.38	0.12	1.01±0.06	0.018	3.32±0.24	0.07	17.08±1.54	0.48	4.15±0.56	0.17
6	Capparis sp.	8	1.13±0.56	0.19	1.08±0.02	0.007	5.37±0.41	0.14	12.41±0.98	0.34	3.19±1.06	0.37
7	Soil	15	1.44±0.52	0.13	0.24±0.02	0.005	7.27±1.74	0.45	8.32±2.61	0.67	6.43±2.01	0.51

Table II : Metals concentrations in Biological samples of Gajner Wildlife Sanctuary, Bikaner, Rajasthan

N=Number of samples, ND= Not detectable, * =Lowest Mean values, # = Highest Mean values, Metal concentration in µg/g (ppm) dry weight.