Heavy Metal Contamination in Ranthambore National Park: Feces as Bioindicators

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Abstract:
Anthropogenic activities near or within the wildlife habitats are threatening the wildlife with exposure to a variety of environmental contaminants. The wildlife horboured in these reserves are at risk of getting expose to automobile exhaust, industrial gases and suspended particulate matters. The world famous Tiger reserve, Ranthambore National Park, Sawaimadhopur, Rajasthan (India) was selected for study. Restrictions on the sampling because of wildlife Protection Act (1972) prevents taking of samples of living tissues to analyse levels of contaminants that wildlife may be carrying and in determining the critical levels that may be detrimental for their survival, well-being and reproduction. Feces of wild mammals one as noninvasive, nondestructive bioindicator of assessing environmental contamination. Feces of wild mammals, vegetation, soil and water of Ranthambore National Park, Sawaimadhopur, Rajasthan showed good concentration of heavy metals (Pb, Cd, Cr, Cu and Zn).

1.0 Introduction:

Pollution of the environment can be determined by means of biological methods with the help of Bioindicators-organisms which give information on the quality vance of their environment. Biomonitoring has many advantages over monitoring of non-biological materials, such as great availability, lost cost, retrospection, no servicing, consideration of synergistic (antagonistic) effects and biological relevance (wittig1993). Animals as accumulative monitors of pollution by heavy metals (which belongs to group of the most dangerous inorganic toxic substances) have some advantages over plants, such as area related results and comparability to man (Wittig, 1993).During the last few decades, heavy metal contamination of biotic component ofenvironment has attracted the attention of many investigators. The main reason of theseresearches based on the heavy metal concentration may have a potential hazard in ourfood chain after a long period of procrastination. Using biological materials in thedetermination of environmental pollution as indicators is a cheap and reliable method.

Now, it is known that heavy metals represent a large group of chemical elements (> 40) withatomic mass > 50 carbon units. Most of heavy metals may be important traceelements in the nutrition of plants,

animals or humans (e.g. Zn, Cu, Mn, Cr, Ni, V), whileothers are not known to have positive nutritional effects (e.g. Pb, Cd, Hg). However all ofthese may cause toxic effects (some of them at a very low content level) if they occurexcessively (Spiegel, 2002). The toxicity of heavy metal depends a great deal on theirchemical form, concentration, residence time, etc (Mielke and Reagan, 1988). Because of theseelements do not decay with time; their emission to the environment is a serious problemwhich is increasing worldwide due to the rapid growth of population, increasingcombustion of fossil fuels, and the expansion of industrial activities (Smodis and Bleise, 2000). Studies have reported concentrations of metals in wild mammals living in highly contaminated area near smelters (Pokorny et al., 2000), chlor-alkali plant (Dżugan et al., 2012), verges of heavily-used highways (Roux and Marra, 2007) and mines or mine waste sites (Beyer et al., 2007).

Ranthmbore National Park, a world famous Tiger reserve is situated 14 kilometers from Sawai Madhopur town. Two newly created sanctuaries Kaila Devi on the northeast and Sawai Man Singh on south are now also part of park. The park derives its name from the Ranthambore Fort situated within its precincts. Park is spread over an area of 392 square kilometers with waterfalls and lakes. The vegetation

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of the park is the tropical dry deciduous and tropical thorn forest types. Important trees include Dhok.,*Anogeissus pendula*, Kadam.,*Anthocephlus cadamba*, Ber., *Zizyphus mauritiana*, Aam., *Mango Magnifera indica*,Ronj., *Acacia leucophloea*,Neem., *Azadirachta indiaca*, Banyan., *Ficus bengalensis,* as well as vast varity of aquatic vegetation like lotus, trapa, nymphaeas. Wildlife of the park belongs to herbivore, omnivore and carnivore category. The herbivores are sambar., *Cervus unicolor*, chital., *Axis axis*, nilgai., *Boselaphus tagocamelus*, rhesus monkey., *Macaca mulatta*, whereas porcupine., *Hystrix indica*, wildboar.,*Sus scrofa*, sloth bear., *Melurus ursinus* are belong to omnivore category and carnivores are tiger., *Panthera tigris*, panther., *Panthera pardus*, jackal., *Canis aureus*, hyena., *Hynea hynea*, fox., *Vulpes vulpues*, jungle cat, caracal. There are various places of historical interest inside the fort and tourist come to visit the sanctuary every day. They commute by car, gypsies, motorbykes etc.

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), Sariska Tiger Reserve, Alwar(Gupta and Bakre, 2012), Desert

National Park, Jaisalmer and Gajner Wildlife sanctuary, Bikaner of Western Rajasthan (Gupta 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. Objective of my investigation is to develop a non-invasive tool for assessment of environmental heavy metal contamination.

2.0 Materials and Methods:
2.1 Sampling Procedure
In the field (Ranthambore National Park) scat sampling was totally opportunistic type. Fresh scat samples of wild mammals of reserve were collected with the help of forest staff from different sites. The sites were selected as near the roadside (disturbed area) and distant roadside (undisturbed area). Samples were brought to the laboratory and kept in freeze for metal analysis. Scat samples of the following mammalian species were collected; Chital., *Axis axis,* Nilgai., *Boselaphus tagocamelus,* Sambar.,*Cervus unicolor,*Rhesus monkey.*, Macaca mulatta,* Porcupine.*, Hystrix indica,* Fruit bat.,*Pteropus giganteus,* Wild boar.*, Sus scrofa,* Tiger.*, Panthera tigris. Panther.,Panthera pardus*. To ascertain the source of contamination water and vegetation (terrestrial and aquatic) samples of this park were also collected. Neem*, Azadirachta indiaca,* Dhok. *, Anogeissus pendula,* Lotus.*, Nelumbo nucifera,* Kadam. *, Anthocephlus cadamba,* Water caltrop.,*Trapanatanus,* Fruit of *T. natanus,*Kumudini., *Nymphoides indica,* Muskgrass.,*Chara Chara,* Khus grass.*, Vetiveria zizanoides,* water spinach., *Ipomoea aquatica.* Another, suspected source of contamination was suspended particulate matter settling on the ground, hence soil samples were also taken from different roadsides of park. Scat, vegetation and soil samples were stored in the plastic zip lock bags and water samples in the sterilized plastic containers.

2.2 Sample treatment

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.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.

2.3 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.

2.4 Calculations

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

Where,

Dilution factor=10

Dry weight of the sample= 0.5 gms

2.5 Statistical analysis

The statistical calculations were based on Ipsen and Feigel's (1970) method. The values are expressed as mean ± standard deviation (S.D.) as well as in standard error (S.E.).

3.0 Results and Discussion:

The fecal matter / scat sample analysis shows the presence of lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), and zinc (Zn) in varying concentrations. Lead was observed in the range of 3.55 to 19.92 ppm d/w, concentration of cadmium was found in the range of 0.79 to 2.18 ppm d/w , chromium was detected in the range of 6.87 to 20.01 ppm d/w copper was observed in range of 6.13 to 28.49 ppm d/w and zinc was estimated in the range of 10.48 to 42.51 ppm d/w in scat samples of park.Maximum concentration of lead was found in scat of *Panthera tigris*i.e. 19.92±2.61 ppm d/w and minimum was in *Hystrix indica* i.e. 3.55±0.75 ppm d/w. Concentration of cadmium was detected maximum in scat of *Sus scrofa* i.e. 2.18±0.05 ppm d/w and minimum was found *Macaca mulatta* i.e. 0.79±0.07 ppm d/w. Maximum concentration of chromium was found in *Panthera tigris* i.e.20.01±3.61 ppm d/w and minimum was in *Boselaphus tagocamelus* 6.87±0.27 ppm d/w. Concentration of copper was detected maximum in *Macaca mulatta i.e.* 28.49±2.31 ppm d/w and minimum was in *Sus scrofa* i.e. 6.13±0.95 ppm d/w. Maximum concentration of zinc was found in *Panthera tigris i.e.* 42.51±1.05 ppm d/w and minimum was in *Panthera pardus i.e.* 10.48±1.80 ppm d/w. (Table 1)The analysis of soil and vegetations (terrestrial and aquatic) indicated that metals i.e. lead, cadmium, chromium, copper and zinc were present in background concentrations in reserve.(Table 2)

Concentration of lead was found maximum in *Trapa natanus*i.e. 17.78±1.87 ppm d/w and minimum was in *Chara Chara* i.e. not detectable. Maximum concentration of cadmium was detected in *Trapa natanus* i.e. 3.8±1.69 ppm d/w and minimum was found *Vetiveria zizanoides*i.e. 0.7±0.02 ppm d/w. Chromium concentration was maximum in *Nelumbo nucifera*i.e. 15.42±2.52 ppm d/w and minimum was in *Nymphoides indica* 4.73±0.93 ppm d/w. Concentration of copper was detected maximum in *Nelumbo nucifera i.e.* 17.24±1.38 ppm d/w and minimum was in *Nymphoides indica* i.e. 3.27±1.16 ppm d/w. Maximum concentration of zinc was found in *Anogeissuspendulai.e.*18.44±1.52 ppm d/w where as in *Nelumbo nucifera, Trapa natanus, Nymphoides indica, Ipomoea aquatica* had not detectable amount of zinc.(Table 2)

Table 1: Metal concentrations in Fecal Samples of Mammalian Wildlife of Ranthambore National Park, Sawaimadhopur, Rajasthan

S.N.	Species	N	Pb (ppm) Mean±S.D.	S.E.	Cd (ppm) Mean±S.D.	S.E.	Cr (ppm) Mean±S.D.	S.E.	Cu (ppm) Mean±S.D.	S.E.	Zn (ppm) Mean±S.D.	S.E.
1.	*Axis axis*	45	7.28±2.26	0.33	1.0±0.03	0.04	10.04±0.80	0.11	20.85±1.40	0.20	20.8±1.86	0.27
2.	*Boselaphustagocamelus*	42	9.84±2.61	0.40	1.04±0.08	0.01	*6.87±0.27	0.04	15.42±1.59	0.24	16.35±1.05	0.16
3.	*Cervus unicolor*	35	8.08±1.83	0.30	1.36±0.09	0.05	8.43±0.51	0.08	20.92±1.90	0.32	15.24±1.10	0.18
4.	*Macacamulatta*	20	5.7±1.13	0.25	*0.79±0.07	0.15	16.66±0.18	0.04	#28.49±2.31	0.51	15.7±1.89	0.42
5.	*Hystrixindica*	15	*3.55±0.75	0.19	1.85±0.06	0.11	14.16±1.98	0.51	9.62±0.58	0.14	18.4±1.32	0.34
6.	*Pteropusgiganteus*	18	7.6±1.15	0.27	1.69±0.05	0.02	12.34±0.56	0.13	10.65±0.20	0.04	22.16±0.59	0.13
7.	*Susscrofa*	26	5.04±1.07	0.20	#2.18±0.05	0.01	13.65±1.19	0.23	*6.13±0.95	0.18	19.13±0.91	0.17
8.	*Pantheratigris*	17	#19.92±2.61	0.63	2.13±0.06	0.012	#20.01±3.61	0.87	22.15±3.16	0.76	#42.51±1.05	0.25
9.	*Pantherapardus*	9	15.04±0.73	0.24	0.50±0.03	0.09	10.01±0.28	0.09	6.48±2.89	0.96	*10.48±1.80	0.6

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

Table 2: Metal concentration in Vegetation (Terrestrial and Aquatic), Water and Soil Samples of Ranthambore National Park, Sawaimadhopur, Rajasthan

S.N.	Species	N	Pb (ppm) Mean±S.D.	S.E.	Cd (ppm) Mean±S.D.	S.E.	Cr (ppm) Mean±S.D.	S.E.	Cu (ppm) Mean±S.D.	S.E.	Zn (ppm) Mean±S.D.	S.E.
1.	*Azadirachtaindiaca*	19	16.14±1.45	0.33	2.27±0.07	0.01	11.0±0.20	0.21	9.95±0.26	0.05	6.35±0.31	0.07
2.	*Anogeissuspendula*	15	15.16±1.96	0.50	1.04±0.03	0.09	6.96±0.28	0.07	12.49±0.41	0.10	#18.44±1.52	0.39
3.	*Nelumbonucifera*	10	12.54±2.78	0.87	1.76±0.12	0.03	#15.42±2.52	0.79	#17.24±1.38	0.43	*ND	-
4.	Fruit of *N. nucifera*	12	3.38±1.16	0.33	2.61±0.04	0.01	9.72±1.3	0.37	10.9±0.88	0.25	4.9±1.9	0.54
5	*Anthocephluscadamba*	18	16.11±0.09	0.12	1.91±0.02	0.66	12.32±1.1	0.82	13.33±1.21	0.72	11.23±0.31	0.43
6.	*Trapanatanus*	15	#17.78±1.87	0.48	#3.8±1.69	0.43	14.57±0.05	0.02	5.20±0.46	0.11	*ND	-
7.	Fruit of *T. natanus*	13	10.81±0.71	0.18	1.3±0.07	0.01	7.37±0.25	0.06	3.43±0.06	0.91	*ND	-
8.	*Nymphoidesindica*	10	16.72±3.46	1.09	2.10±0.17	0.05	*4.73±0.93	0.29	*3.27±1.16	0.36	*ND	-
9.	*CharaChara*	15	*ND	-	1.89±0.05	0.11	8.9±1.1	0.28	9.7±2.04	0.52	6.3±1.4	0.36
10.	*Vetiveriazizanoides*	10	14.38±1.15	0.36	*0.7±0.02	0.52	5.07±0.09	0.02	10.98±1.03	0.32	15.09±0.73	0.23
11.	*Ipomoea aquatica*	11	14.0±1.72	0.57	0.87±0.16	0.05	8.28±1.78	0.59	12.85±0.17	0.56	*ND	-
12.	Water	13	10.26±0.35	0.09	0.35±0.02	0.46	2.36±0.02	0.93	7.54±1.26	0.34	9.3±1.06	0.29
13.	Soil	10	8.20±0.44	0.13	0.4±0.03	0.09	7.81±2.17	0.65	6.29±0.6	0.18	17.31±1.12	0.33

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

Soil and water samples also showed background concentration of lead, cadmium, chromium, copper and zinc. Concentration of lead was 8.20±0.44 ppm d/w, cadmium was 0.4±0.03 ppm d/w, chromium was 7.81±2.17 ppm d/w, copper was 6.29±0.6 ppm d/w and zinc was 17.31±1.12 ppm d/w in soil samples of reserve. Whereas concentration of lead was 10.26±0.35ppm d/w, cadmium was 0.35±0.02 ppm d/w, chromium was 2.36±0.02 ppm d/w, copper was 7.54±1.26 ppm d/w and zinc was 9.3±1.06 ppm d/w in water samples of reserve. (Table 2)Heavy metal concentrations were found in background amount in the biological samples collected from Ranthambore National Park. Reserve

have little vehicular movement and there is no urban settlement nearby. This is reason that most of the biological samples of this tiger reserve 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 as well as studying an interactions between toxic and essential metals. Thus analysis of scat has distinct advantages that it indicates gross exposure, does not involve disturbing and killing the animals and monitoring of exposure to contamination at 24 hours interval. Only disadvantages is that since the quantum and routes of exposure are difficult to pinpoint fecal concentration can't be used as bio-sensor but as bio-indicator only. 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. This method is simple, non-continuous and relatively inexpensive on an individual basis.

4.0 Conclusions:

Our results shows that fecal matter can use as good bio-indicator because -
1. It indicates the gross metal exposure.
2. It provides a less expensive method as bio-indicator.

3. Better means of assessing long-term trends in pollution or other forms of environmental change.
4. This method is completely non-invasive one to conserve the wildlife.

5.0 Acknowledgement:

References:

1) Beyer, W.N., Gaston, G., Brazzle, R., O'Connell, A.F. and Audet, D.J. (2007): Deer exposed to exceptionally high concentrations of lead near the Continental Mine in Idaho, USA. *Environ Toxicol Chem.,* 26:1040–1046.

2) Clark D.R. Jr., Richard K.L.V. and Merlin D.T. (1982): Estimating pesticide burdens of bats from guano analysis.*Bull.Environ. Contam. Toxicol.* 29: 214-220.

3) Dżugan,M, Zielińska,S., Hęclik,J., Pieniążek,M,and Szostek, M. (2012): Evaluation of heavy metals environmental contamination based on their concentrations in tissues of wild pheasant (Phasianus colchicums L) *Journal of Microbiology,Biotechnology and Food Sciences*, 2 (1): 238-245.

4) Gaumat, V. and Bakre P.P. (1998): Mammalian dung as a bioindicator of heavy metal contamination.*Proc. Acad. Environ. Biol.*,7(1): 99-102.

5) Gaumat, V. and Bakre P.P. (2001): Metal contamination in mammalian fauna of Keoladeo National Park, Bharatpur (India). Environment and Agriculture: Biodiversity Agriculture and Pollution in South Asia. 577-580.

6) Goyer R.A. (1986): Toxic effects of metals. In: casarett and Doull's Toxicology.The Science of Poisons. (ed) Klaassen, C.D. *3rd Ed. Macmillan Publishing Company, 582-653.*

7) Gupta, V. and Bakre P.P. (2012): Metal contamination in mammalian fauna of Sariska tiger reserve, Alwar, India.*Journal of Ecophysiology and Occupational Health,* 12: 43-48.

8) Gupta V. (2012):Mammalian Scat as a Bio-indicator of Heavy Metals Contaminationin Western Rajasthan, India.*International Journal of Scientific and Research Publications*, 2(12):1-7.ISSN 2250-3153

9) Ipsen, J. and Feigel, P. (1970): In Bancrofts Introduction to Biostatistics. *2nd Ed. Harper and Row Publisher. Inc.; New York*.

10) Kjellstrom, T. Borg, K. and Lind, B. (1978): Cadmium in feces as an estimator of daily cadmium intake in Sweden, *Environ. Res*, 15: 242-251.

11) Klaassen, C.D. (1976): Biliary excretion of metals. *Drug. Metab. Rev*, 5: 165-96**.**

12) Leonzio, C. and Massi, A. (1989): Metal bio-monitoring in bird eggs: A critical experiment. *Bull. Environ. Contam. Toxicol.*, 43: 402-406.

13) Littele, P. and Wiffen, R.D. (1977): Emission and deposition of petrol engine exhaust Pb-I, Deposition of exhaust Pb to plant and soil surfaces.*Atmos. Environ.*,11: 437.

14) Littele, P. and Wiffen, R.D. (1978): Emission and deposition of lead from motor exhaust II. Airborne concentration, particle size and deposition of lead near motorways.*Atmos. Environ*., 12: 1331.

15) Mielke, H.W. and Reagan, P.L. (1988): Soil as an Impact Pathway of Human Lead Exposure.*Environmental Health Perspectives*, 106(1): 217– 29. ISSN 0091-6765

16) Petit, M.G. and Altenbach, J.S. (1973): A chronological record of environmental chemicals from analysis of stratified vertebrate excretion deposited in a sheltered environment. *Environ. Res.,* 6 (3): 339-343.

17) Pokorny B, Ribarič-Lasnik C. Lead, cadmium and zinc in tissue of roe deer (*Capreolus capreolus*) near the lead smelter in the Koroška region (northern Slovenia) *Bull. Environ. Contam. Toxicol.* 2000;64:20–26.

18) Reidinger, R.F. Jr. (1972): Factors influencing Arizona bat population levels, Ph.D. Thesis, Univ. Arizona, Tucson, 172.

19) Roux, K. E. and Marra P. P. (2007):The Presence and Impact of Environmental Lead in Passerine Birds Along an Urban to Rural Land Use Gradient. *Arch. Environ. Contam. Toxicol*., 53: 261–268.

20) Sileo, L. and Beyer, W.N. (1985): Heavy metals in white-tailed deer living near a zinc smelter in Pennsylvania.*J. Wildl. Dis,.* 21: 289-296.

21) Smodiš, B. and Bleise, A. (2000): Biomonitoring of Atmospheric Pollution, Proceeding of International Workshop on Internationally harmonised approach to biomonitoring trace element atmospheric deposition. pp. 143–150, ISBN 92–0–100803–1, Portugal, 28 August–3 September, 2000.

22) Sobel, A.E. Gawron, O. and Kramer B.(1938): Influences of vitamin D in experimental lead poisoning.*Proc. Soc. Exp. Biol. Med.,* 38: 433-435.

23) Spiegel, H. (2002): Trace Element Accumulation in Selected Bioindicators Exposed to Emissions along the Industrial Facilities of Danube Lowland, *Turkish Journal of Chemistry*, 26(6), 815 – 823,ISSN 1300-0527

24) Way, C.A. and Schroder, G.D. (1982): Accumulation of lead and cadmium in wild population of the commensal rat, *Rattusnorvegegicus. Arch. Environ. Contam. Toxicol*. 11: 407-417.

25) Williamson, P. and Evans, P.R. (1972): Lead: Leavels in roadside invertebrates and small mammals.*Bull. Environ. Contam. Toxicol.,*8: 280-288.

26) Wittig, R.(1993): General Aspects of Biomonitoring Heavy Metals by Plants. In: Plants as biomonitors - Indicators for heavy metals in the terrestrial environment,B. Markert, (Ed),.3-27, VHC, ISBN 3527300015, Weinheim (1993).