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Animal Hair as Geographical Region Indicator in Wildlife Forensic Crime Investigation

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Abstract

This work presents a study of the feline hairs as a geographical region indicator from detection of elements for forensic geographical identification. The animals from Felidae family were selected for the study and elemental analysis was done on three selected species i.e. *panthera leo, panthera pradus fusca and panthera tigris* from different geographical areas of Gujarat state using Scanning Electron Microscope-Energy Dispersive Spectra (SEM-EDS), and Energy Dispersive X-Ray Fluorescence (EDXRF). After the entire elemental analysis, the results have revealed that the animals of all geographical regions have shown to have different levels of metallic contents from nil to the highest for Sulfur, Calcium , Iron, potassium and manganese but barium has shown a significant demarcation among animals of all three species and observed only in the animals from Zunagarh region up to 9.879%. Which is further confirmed by the presence of these metals from the soil and water sample analysis of the subsequent region and found to be absent in Indroda park and Kankaria region. Our study provides a geographical bio-marker in forensic wildlife investigation related to the corresponding areas.

Keywords: Wildlife forensic; Hair examination; Elemental analysis

Introduction

Wild life plays an important role in eco balance, In fact there are more economical and social importance associated with the wild animals. The use of hair for studying environmental factors and work culture have been explored by many researchers from all over the world, some researchers have established a purest association between hair and the environment[1-3]. Microelements play vital role in the functioning of all living organisms and also in pathological status. Cu, Zn, Mn, Fe ,Ca, Ba and many other elements are necessary for animals and in humans [4]. One of the substrata accessible for investigation of these elements are hairs. Feeding habits, external environment and metabolism associated processes leads to the origin of these elements in hair. These elements have been selected for biomonitoring worldwide in global environment monitoring system (GEMS) of the united nations environment programme in EPA 2001[5]. In recent years, elemental study of the hair composition has become the subject of interest for forensic investigation of chronic toxicity due to heavy metals, toxicants, ecological aspects and clinical aspects [6-12].

Many studies have been done on hair for elemental analysis has revealed about the level of trace elements in hair reflects, the degree of environmental concentration like soil, water, food and metabolism. Higher levels of these elements in the environmental concentration like soil, water, food intake and metabolism lead the organism for strong natural selection for tolerance. As environmental contaminants are of great importance in forensic analysis in cases where hair is used as an evidence since last century [13]. The concentration of these elements varies between each geographical region which leads to different levels for accumulation of these elements in hairs. Animal hairs show significant variation in the levels of these elements then humans because of their extended exposure to soil contaminants through feeds [1,14] in the contemporary studies on forensic science, clinical and toxicological studies involves elemental analysis of hair has been done with the specific interest on the chronic toxicity among animals due to heavy metals of elements present in the soil for longer durations [15,16]. Hairs of *panthera leo* (Lion), *panthera pradus fusca* (Leopard) and *panthera tigris tigris* (Tiger) were used in the present study for the production of forensically important geographical markers from elemental profiling, soil and water samples. In the present study an attempt has been made to correlate the environmental conditions with that of hair with the elemental analysis on them would be ultimately useful for the determination of the geographical area or the inhabitation of the animals.

Materials and Methods

Reagents

Absolute alcohol, SDS, HNO_3, H_2O_2, Rectified Spirit used were analytical grade reagents procured from Hi-Media and Merck specialties. Carbon tape, aluminum adhesive and gold palladium coating material was taken from ZEISS SEM supplies.

Sample collection

Hairs of lion, leopard and tigers were collected from Indroda nature park, Gandhinagar (23°11'32.70"N, 72° 39'03.04"E); Kamala Nehru Zoological Garden Kankaria, Ahmedabad (23°00'34.11"N, 72°35'58.17"E); Sakkarbaug Zoo (21°32'27.05"N, 70°28'06.53"E), Wildlife Rescue Center Junagadh (21°32'56.82"N, 70°28'23.44"E) wide letter No. WLP/28/B/4651-54/2012-13 from the Office of the Principle Chief Conservator of Forests and Chief Wildlife Warden, Gujarat State. 10 hair of each of 90 animals were taken from the cage and enclosure using forceps with gloves to avoid contamination and were packed in zip-lock bags followed by paper envelops. Soil and water samples of the same were collected from 30 different sites from the area selected for the present study. Geographical locations were

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Sample preparation

Hair samples were washed with absolute alcohol followed by washing in 2% SDS, rinsed, dried in oven at 40 degree for about 24 hours followed by packing in fresh zip-lock bags and stored at 0°C till further analysis. For EDXRF hair samples were dissected into 2 mm pieces and placed on sample holder using forceps. For EDS hair samples were dissected in pieces of 5 mm size and places on sample holder followed by Gold-Palladium coating at 5 milibar vacuum and 5 mA current.

The systematic examination using various instruments resulted in detection of the elements with their corresponding quantities. These have shown the constituent concentrations of the elements that are present both in soil samples as well as in hair samples.

EDXRF analysis

Samples were analyzed under Energy Dispersive X-Ray Fluorescence Spectrometer (EDXRFS) by Shimdzu EDX Series EDX-720/800 HS with 10 mm collimeter was used for the elemental analysis. The instrument was calibrated using standard calibration metal cubes provided with the instrument and then the hair samples were placed for analysis. Readings of the elements were taken on two channels, first from sodium to scandium whereas the second channel by Titanium to Uranium [17,18]. Comparative and percentage analysis was observed for Sulfur, Iron, Calcium, Copper and barium. The analytical equipment was tested with the both interlaboratory and intralaboratory controls before use.

SEM-EDS analysis

The samples were examined under Scanning Electron Microscopy [SEM] using Zeiss EVO 18 Special edition for the elemental analysis using gold palladium coating for 120 seconds at 5 mA current for plasma. The SEM-EDS was taken with WD of 9.5 mm gun beam on 20kV using Energy dispersive Spectrum detector provided by Oxford Instruments and the elements were analyzed using Dot-Id setup for the elements S, Cu, Fe, K, Ca and Ba.

Results

For getting the clarity of the micro and macro nutrients that are essential for the smooth functioning of the cells and the system in general for any living organism. To achieve this, the soil samples, those have been collected from the ambience of the specific animals that are under study were examined for the quality and quantity of various elements which include the macro and micro nutrients. These samples have been examined for barium, calcium, iron, silicon, titanium and manganese. The soil samples from Indroda and Kankaria region have been examined. Among the soil samples of Indroda region showed no trace of barium, silicon. There were predominantly high concentrations of calcium and iron, less significant quantities of manganese and titanium were noted.

From Table 1 and Figures 1 and 2, it is possible to establish that soil samples from Junagadh region showed significantly higher concentrations of the barium and silicon. These have been much consistent and uniform in all the sample groups. In case of calcium and iron, it has been shown that there was least significance in the difference in the concentration of these two elements. The same types of the results were available for titanium and manganese as they also did not show much of the significant differences.

In case of hair samples from Indroda and Kankaria Region, it was found to have contained no trace of barium at all, so barium does not have any presence both in the ambient atmosphere and so also in case of hair samples as shown in Figure 3.

In case of iron and calcium that was less significant concentration of these metals was noted. Sulfur and potassium were also found to have in different concentrations in hair samples. This also could not be taken into consideration for the co-relation of elements present in ambient (soil, and the hairs) as shown in detail in Table 2.

In case of Junagadh region, presence of barium was found in significant quantities. The same kind of significant levels of concentrations of barium were observed in the hair samples of animals of this region shown in Figure 4.

The major elements such as iron and calcium were found in higher concentration levels, both in the soil as well as in hair samples of that region. This may not be taken for consideration as they don't show much of significant differences which are showed in detail in Table 3.

Comparative analysis of different elements i.e. sulfur is found more prominent in animals from Indroda and Kankaria region whereas iron has shown relatively slight low concentration levels in hairs of animals from Indroda region than in Junagadh region and same observations were observed in elemental profile of the soil samples for iron as shown in Figures 5 and 6 respectively.

Group No.	Barium (Ba)	Calcium (Ca)	Silicon (Si)	Iron(Fe)	Titanium (Ti)	Manganese (Mn)
S 1	0	76.187	0	19.025	2.09	0.355
S 2	0	75.537	0	19.254	2.2	0.383
S 3	0	77.173	0	17.93	2.161	0.335
S 4	0	73.785	0	20.512	2.573	0.42
S 5	0	75.597	0	19.203	2.287	0.382
S 6	2.874	18.561	17.998	57.127	3.872	1.116
S 8	2.67	15.956	18.874	58.773	4.012	1.152
S 9	2.689	18.071	18.632	55.914	4.169	1.17
S 7	2.696	16.008	17.813	57.368	3.143	1.148
S 10	2.699	15.425	17.066	59.448	3.133	1.047
S 11	3.078	15.721	18.787	56.92	3.217	1.148
S 12	2.458	15.195	18.125	57.857	3.163	1.024

*Group S-Soil, S1-S5 are from Indroda Region and S6-S12 were from Junagadh Region
**Trace amount of Cu, K, Mn, Zr, Sr, Ti, V were observed during the analysis but showed no significance

Table 1: Showing elemental composition percentage analysis of the soil samples from Indroda and Kankaria Region and composition of samples from Junagadh region.

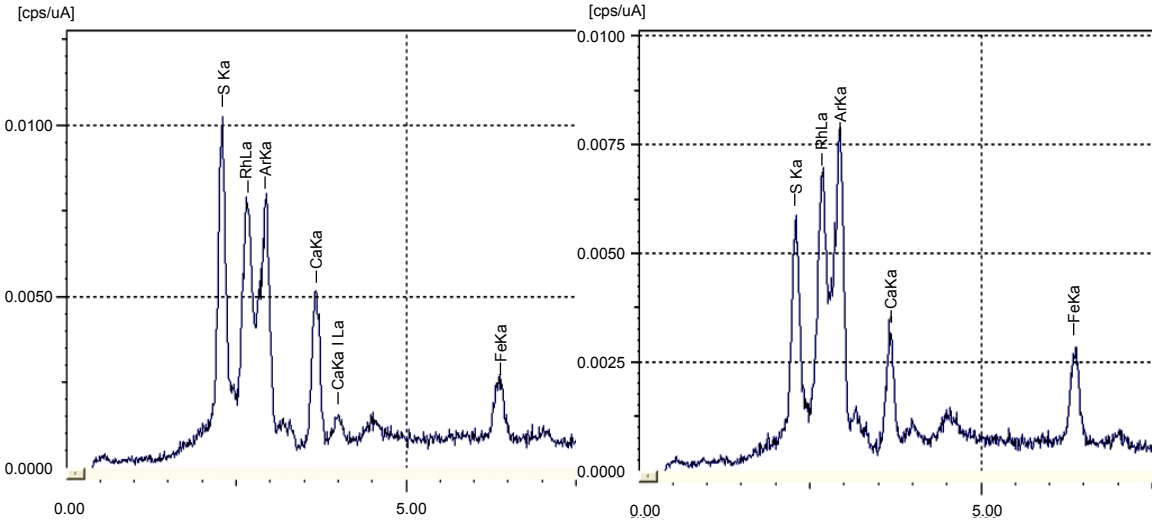

Figure 1: Elemental profile of soil from Kankaria and Indroda Nature Park region.

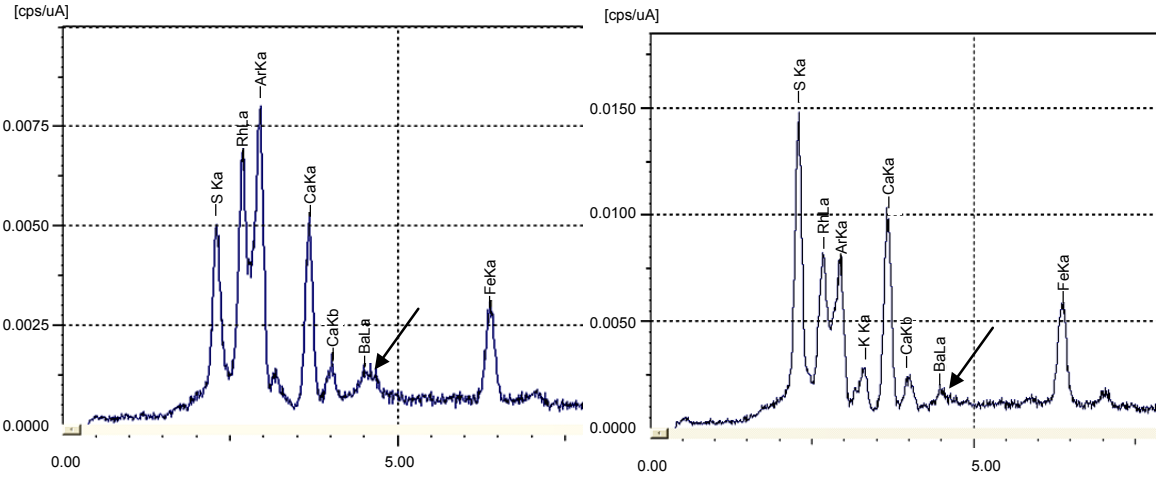

Figure 2: Elemental profile of soil from Junagadh Zoo and Wildlife Rescue Centre region.

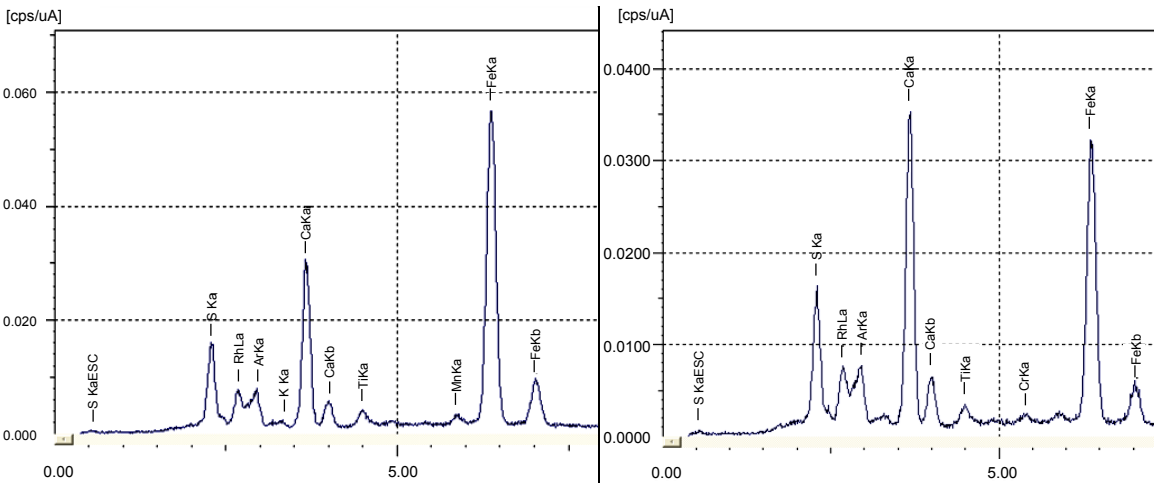

Figure 3: Shows elemental profile of hair samples from Kankaria and Indroda region.

Group ID/ No.	Sulfur (S)	Calcium (Ca)	Potassium (K)	Iron (Fe)	Barium (Ba)
LE 1/01	41.595	36.284	22.369	3.444	0
LE 2/02	50.621	36.22	18.23	2.371	0
LE 3/03	44.876	29.488	15.497	5.562	0
LE 4/04	55.408	34.207	16.93	3.011	0
T 1/05	39.435	44.71	21.345	3.847	0
T 2/06	46.335	28.881	22.895	1.889	0
L 1/07	42.701	24.094	18.023	2.078	0
L 2/08	56.401	38.274	19.978	5.325	0
L 3/09	43.238	22.685	28.979	4.005	0
L 4/010	31.688	47.839	16.833	1.121	0

*Group LE-Leopards, L- Lion and T- Tiger
**Trace amount of Cu, Mn, Zr, Sr, Ti, Si, V were observed during the analysis but showed no significance

Table 2: Shows elemental composition of hair Samples from Indroda and Kankaria region.

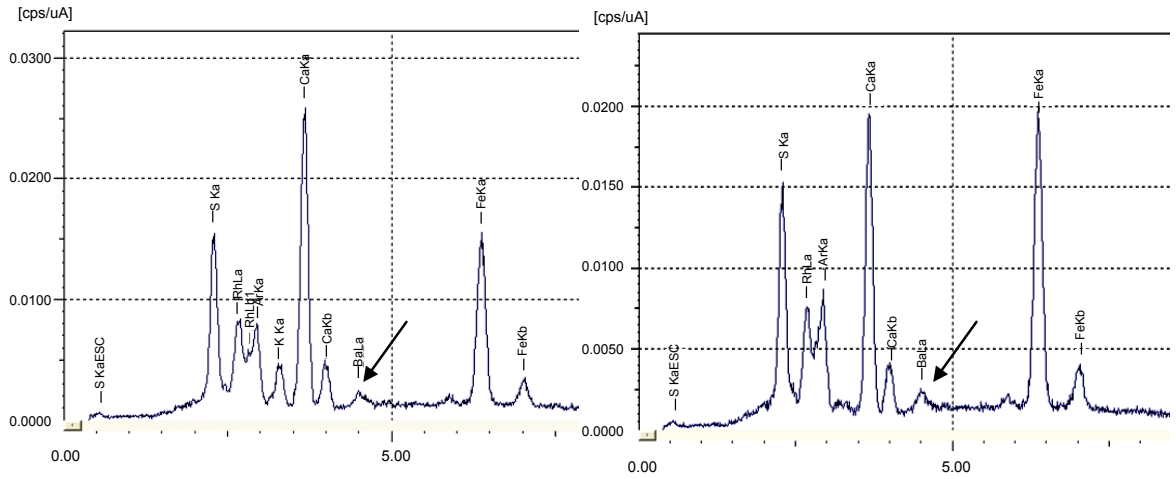

Figure 4: Shows elemental profile of hair samples from Junagadh Zoo and Rescue Centre.

Group Id/ No.	Berium (Be)	Sulphar (S)	Calcium (Ca)	Iron(Fe)
L 5/01	6.597	19.951	71.271	1.863
L 6/02	9.114	51.192	95.286	3.146
L 7/03	7.979	46.394	26.804	2.794
L 8/04	7.513	33.204	30.564	5.687
L 9/05	6.392	55.628	30.871	6.699
T 3/06	3.991	38.158	49.225	4.57
T 4/07	4.144	15.319	54.077	6.035
T 5/08	6.569	24.863	45.976	11.024
T 6/09	9.97	26.72	42.997	19.936
T 7/010	7.22	23.407	43.484	21.804
LE 5/011	8.489	60.98	23.819	5.51
LE 6/012	7.82	30.836	36.895	18.845
LE 7/013	6.729	45.155	31.785	9.049
LE 8/014	7.949	32.332	32.705	19.425
LE 9/015	7.59	23.689	49.973	14.311
LE 10/016	9.879	18.434	45.743	22.823
LE 11/017	9.481	31.788	40.726	16.904
LE 12/18	6.47	30.893	40.512	18.121
LE 13/019	6.653	31.977	31.977	4.42
LE 14/020	9.024	19.568	46.47	21.758

**Group LE-Leopards, L- Lion and T- Tiger
*Trace amount of Cu, K, Mn, Zr, Sr, Ti, Si, V were observed during the analysis but showed no significance

Table 3: Shows elemental profile of Hair Samples from Junagadh Zoo and Wildlife Rescue Centre Junagadh.

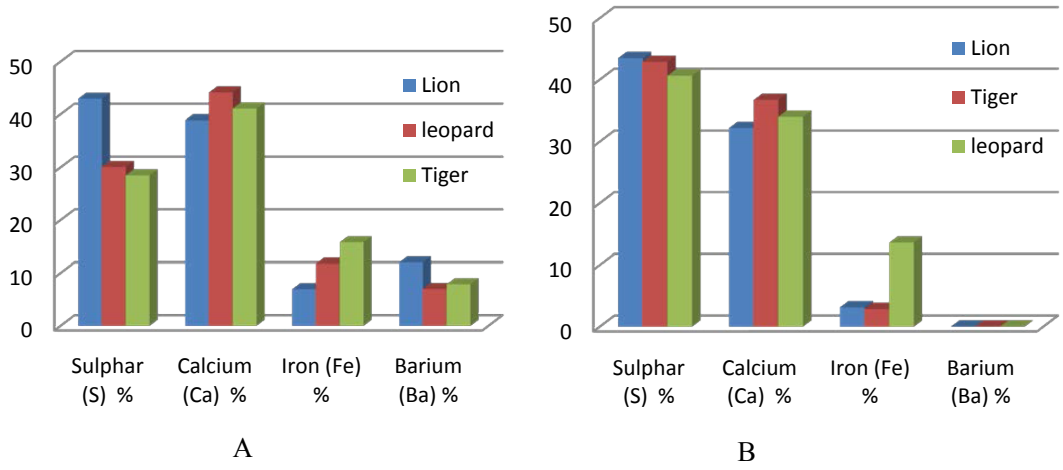

Figure 5: (A) Showing elemental analysis of hairs from Zunagarh Zoo and Wildlife Rescue Centre, Zunagarh and (B) Showing elemental analysis of hairs from Indroda Nature Park and Kamla Nehru Zoological Garden Kankaria.

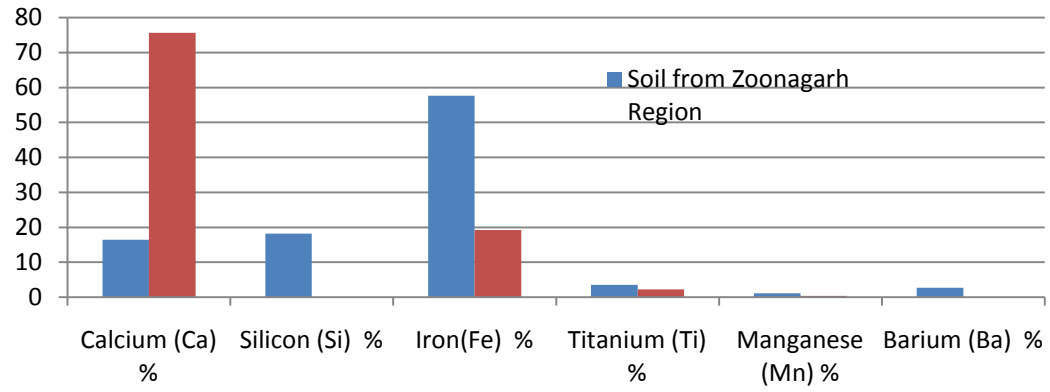

Figure 6: Showing elemental analysis of soil from Zunagarh Zoo and Wildlife Rescue Centre, Zunagarh and Indroda Nature Park and Kamla Nehru Zoological Garden Kankaria.

Discussion

The concentrations of the various elements in the soil and hair samples are in concurrence with the given conditions. In other words, the quantity of the elements present in the hair samples are in perfect concurrence with that of the soil samples. This will be more indicative and informative about the place of the animal inhabitation purely based on the hair elemental analysis. In the same way the absence of the elements in the ambience i.e. the soil samples were found to be absent in the hair samples. This denotes the geographical location/inhabitation of the animal with the aid of the hair samples through the elemental analysis. The results of this study is in concurrence with the studies done on the hair samples of human beings in industrialized area by Ramakrishna et al. [1]. In that study also the elemental concentrations in the hair samples who were exposed to the industrialized areas, the levels were in perfect tender with the concentration of such elements in the ambience. Dietz et al. [8] and Sakuma et al. [19] showed the results in which the elemental concentrations in the hair samples of the individuals were found to be similar to the results of the present study in which the elements were detected with the ambience and soil. Though, these studies involve the presence of elements such as sulfur, calcium, iron, titanium, manganese, copper, cadmium, these elements may have the persistence in the endogenous system [19]. But the present study revealed the element like as barium which is remarkably significant due to its absence in vital systems and can be used to establish relation between both the ambience and the hair samples.

The findings and the results of the work is purely an outcome of the study done in the Gujarat region. These results are open for the future researchers to throw more light in this area and enhance the results by extending this to larger geographical areas. The presence of the elements finds more importance so far as the geographical area is concerned. This may be well correlated among the soil and hair samples.

Conclusion

Based on this study, it is possible to deduce that the elemental analysis of hair would be a significant indicator of geographical origin or inhabitation of animals just based on the hair examination without the invasion of the tissue of such animals under study.

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