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APPLICATION OF ANALYTICAL TOOLS IN THE CONSERVATION OF THE AFRICAN ELEPHANT (*LOXODONTA AFRICANA*)

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ABSTRACT

There is a great need to characterize African elephant ivory for controlling poaching and implementation of national and international laws. In this study, Ivory samples (117) from South African and Namibian sources were analysed for elemental presence using UV laser magnetic sector inductively coupled plasma-mass spectroscopy (ICP-MS). A total of forty five elements were detected. The analysis of variance was carried out over 13 regions. The concentration of elements showed statistically significant differences ($p< 0.0001$) between ivory obtained from different regions. The discriminant analysis was employed to determine whether the existing data can be correctly classified into their region of origin. The data suggested that various elements from distinct ecological regions can present in different concentrations. The relationship of these elements may lead to the establishment of characteristic elemental patterns, unique for each ivory source. This study indicates that chemical analysis of ivory could be beneficial in tracing the source of illegal ivory. This knowledge may play a role in the conservation of the elephant herds.

Key words: Elephant ivory; composition; Environment; laser ablation ICP-MS.

INTRODUCTION

The diversity of plant and animal life is arguably the Africa's greatest natural resource and certainly one of South Africa's greatest resources. African (*Loxodonta africana)* elephants have been hunted for the ivory. Researchers speculate that the African elephant declined from 1.2 million to 600 000 in one decade (Barnes *et al*., 1999; Blanc *et al*., 2003).

Protection of elephant, as well as of game in general, by their own personnel in the different sanctuaries would no doubt be the most effective conservation strategy. However, when suspicion is raised about the legality of a sample of ivory and there is a need to discover whether it represents an illegally harvested lot of ivory, a methodology that can identify the region or sanctuary of origin could be a key factor in the control of poaching.

A number of studies concentrating on the chemical analysis of dentine (i.e. ivory) have been published (Raubenheimer *et al*., 1998; Van der Merwe and Lee-Thorp 1990; Vogel *et al*., 1990). The aim of these studies were twofold viz. the examination of analytical methodologies applied to dentine as well as the establishment of a database consisting of the information on the organic as well as the inorganic content of ivory from different regions. A well ordered collection of such data would provide a valuable resource for monitoring the changes in the environment over time due, for example, to pollution as reflected in the composition of the dentine. Furthermore, the above-mentioned study indicated that there are clear differences in the concentrations of the different elements in ivory from different regions. The inorganic composition of dentine reflects to a considerable extent, the composition of the elephant's diet (Posner and Tannenbaum 1984). This composition remains stable in the dentine after it has been formed, unlike bone and soft tissue that undergo temporal change. As the geo-chemical composition of the various regions of South Africa are quite different, the chemical analysis of a particular sample of ivory might indicate its origin and help to identify whether it was illegally harvested or not.

In the above-mentioned study, some seventeen elements were identified in ivory that reflected the chemical nature of the environment where the elephants had roamed. The differences in concentrations of the elements provided some evidence that such analyses could help to trace the regional origin of the ivory.

Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) has seen increasing use as an analytical tool to detect trace element concentrations in a variety of materials. Its ability to detect specific areas has been useful in the analysis of temporal variation in the elemental composition of human teeth (Lochner *et al*., 1999).

The present study was conducted with an aim of developing an improved method of identification. Modern mass spectrometry combined with laser technology was employed. A discriminant function based on the available data with the view to using the function for identifying the provenance of new samples was developed.

MATERIALS AND METHODS

Collection and chemical analysis of elephant ivory samples: Fragments of ivory of African elephant *(Loxodonta africana*) were obtained from different regions of Southern Africa through the National Parks Board of South Africa. The sources of ivory samples subjected for analysis are listed in Table 1. Nine samples were analysed per ivory source and therefore 117 samples were analysed in total. All ivory was obtained from animals which died of natural causes or as part of the culling procedures employed in the respective regions. Samples dimensions were typically in the range of (8+/-4) x (6+/-3) x (3+/-2) mm. The analysis was performed without additional sample preparation.

Equipment: A Finnigan double focusing magnetic sector ICP-MS with an UV (266 nm wavelength) laser ablation system for direct micro-sampling and measurement of the elemental (isotopic) presence in the ivory samples was used. Isotopes of major, minor and trace elements were monitored. The laser beam diameter used for ablation on discrete spots of the sample surface was approximately 120 µm. Optimal instrumental conditions for the analysis of ivory samples and elements were established after necessary investigations. The single transverse mode output has been Q-switched to give a pulse width of 8 ns and a maximum operating energy of 3.5 mJ pulse^{-1}. The beam profile is Gaussian. The plasma was maintained at ground potential. The ions produced were extracted and accelerated into the mass analyser which is a double is focusing electromagnetic sector instrument based on a reverse Nier-Johnson geometry. Ion detection was by secondary electron multiplier, the ions hit a conversion dynode and the electrons produced were amplified in an electron multiplier which can operate in either counting or analog mode. Data were calibrated to parts per million using the NIST glasses, with ^{43}Ca as an internal standard (Dolphin *et al*, 2005).

Analytical procedure: The ivory specimens were placed in the ablation cell directly from their storage vessels using Teflon plated tweezers. The laser beam was focused on to the individual sample surface using a colour CCD camera and appropriate optical lenses. A period of 30 seconds pre-ablation was used to avoid possible contribution from surface contaminants. An acquisition time of approximately 60 seconds was applied. Each sample was analysed independently on three sites along the sample surface. The ablated small amount of material was transported by a flow of argon gas to a high temperature plasma. The generated isotopic ions, proportionate to the respective elemental concentrations in the sample, were separated and measured by a double focusing magnetic sector mass spectrometer. The collected intensity data were statistically processed using the analysis of variance (SAS/STAT: PROC GLM) to create characteristic patterns and means for reliable differentiation of the ivory sources. The discriminant analysis (DISCRIM procedure, SAS/STAT) which is a multivariate analytical method was used to fingerprint the samples from different regions. Principal component analysis (PROC FACTOR SAS/STAT) was used to find an aggregate of the variables in a data matrix such as is dealt with here.

RESULTS AND DISCUSSION

Analysis of Variance: The ANOVA was carried out for each of the elements over thirteen regions. Statistical analysis showed the differences between the respective elements in the geographical locations indicated to be significant ($p < 0.0001$) (Table 2). It was also evident from the results that different ivory sources contain elements in different concentrations (e.g. Calcium, Lithium, Boron, Fluorine, Silicon, Manganese, Zinc, Barium, Arsenic, Chromium, Mercury, Lead, Selenium, Molybdenum, Nickel, Copper, Thorium, Uranium, Antimony, Titanium and others). Lithium concentration in the Caprivi-Namibia sample was several times higher than any of the other ivory sources. Boron on the other hand, in one of the Addo samples was the highest of all and greatly different from another Addo ivory source.

The R-squared value represented a correlation coefficient squared. This statistic reflected the extent to which the nine values were correlated per ivory source. A value of $R^2 = 1$, would mean that the rank of the nine values would be identical over the thirteen regions. Generally, most of the three values were good (0.7 to 09). Values of less than 0.7 indicated that the nine values of an element are not providing a similar ranking. $R^2 = 0.3$ or 0.4 are poor and clearly, the analysis of these elements should be checked.

The P-values represented the probability that the values of an element are basically the same over all the regions, thus P=0.0001 meant that the probability that they are the same is exceedingly small, and therefore the probability of their being different was large. Perusal of these values showed that some of the elements over the regions are beset with considerable noise to the extent that they are virtually random.

A study of these values would possibly be of value in the following contexts:

a) Developing a database about the level of elements in the different regions, as well as information about those regions that differ significantly from others.

b) A check on the analytical techniques and/or on the ivory samples for those elements which do not provide repeatable results.

Multivariate analyses of 45 elements by 13 regions: The stated aim of the analysis of these ivory samples was to 'fingerprint' the samples from the different regions.

This means being able to find a pattern in the elements that will allow the regional origin of a sample to be determined. The establishment of a method whereby the samples may be so classified may also be useful for classifying a new sample with unknown provenance. Discriminant Analysis is a multivariate analytic method specifically applicable to this problem. It is designed to aggregate all the elements (variables), by fitting a linear function, and then determine whether the existing data can be correctly classified into their region of origin. The first step was to determine which of the elements are most likely to be useful for classification. A stepwise discriminant analysis procedure was run on the full set of data and four different criteria of element inclusion were used in four separate runs. In all these cases the same 27 elements emerged as contributing the most to discriminating between the samples from the different regions. The number of elements was therefore restricted to these 27, and this data was subjected to an analysis which is designed to fit a linear equation incorporating all the 27 elements. The coefficients of this function should be such that when the data from the sample is entered into the function, it correctly classifies the sample into the region from where it originated. As there are nine replicates from each region, the analysis goes further and randomly chooses a sample to be left out of developing a new function, and then uses this sample to validate the function by testing whether it classifies it correctly.

This analysis provides the following results: Seven of the regions classified correctly viz. 1-Siberia, 11-Kruger, 2-Addo, 4-Kavango, 5-Caprivi, 8-Koins, 9-Hestria
The following regions showed 80% of samples correctly classified and 20% of samples incorrectly classified under 13-Kruger: 10- Tembe, 12-Kruger, 13-Kruger, 3-Addo, 6-Olifants, 7-Moeder.

Attempts to improve the classification process:
1. Further discriminant functions were developed that included some elements that had been left out in the previous run. These attempts all resulted in more misclassifications.
2. 2. Removal of 13 –Kruger from the analysis: **resulted in all cases being classified correctly.**

Principal Component analysis: A Principal Component Analysis was carried out for each region separately using all 45 elements. Two PCs were obtained for each region. PCA exploits the correlations between the nine samples to fit a linear function over all the elements such that the first linear function must account for the most variation in the sample whilst the next one accounts for the rest. The first PC (PC1) then represented the stable pattern of elements in a region whilst PC2 represented the noise. The percent of variance accounted for by each of the regions is given below:

Regions which classified correctly with region 13 included:

1-SIBERIA	76	24
2-ADDO	60	40
4KOVANGO	61	39
5-CAPRIVI	68	32
8-KOINSE	57	43
9-HESTRI	61	39
11KRUGER	66	34

Regions which classified incorrectly:

10-TEMBE	54	48
12-KRUGER	57	43
3-ADDO	59	41
6-OLIFANT	64	36
7-MOEDER	57	43

It appears that the regions which classified correctly did show a rather more clearly defined pattern of element behaviour, which is a higher percentage for PC1, than those that did not. Results from the nine individual analyses per ivory source indicate a rather homogenous elemental distribution. This is indicated by the R-squared values that are between 0.7 to 0.9 (Table 2). A stepwise discriminant analysis procedure was used to determine which of the elements in the 45 are most likely to be useful for classification. Twenty seven (27) elements emerged as contributing the most to discriminating between the samples from the different regions. The successful classification of all cases resulting from the removal of 13-Kruger may indicate that: (a) this region represents such a wide ecological diversity with the result that many samples are recognised as belonging in this region. (b) The samples from this region diverge widely.

The results showed that various elements present in different concentrations and their proper association or relationship may lead to the establishment of characteristic elemental patterns unique for each ivory

Table 1: Ivory sources

No.	*Source*
1	Siberia
2	Addo
3	Addo
4	Kavango Namibia 18
5	Caprivi Namibia 16
6	Olifantsbad Namibia 2
7	Moederib Kaokoland Namibia 2
8	Koinseb Namibia 6
9	Hestria namibia 11
10	Tembe 3
11	Kruger 4
12	Kruger 6
13	Kruger 3

Table 2: Summary of R-square and P-values of 45 elements derived from 13 regions

Element	R- Square	P-Values
Ca	0.838425	< 0.0001
Li	0.974795	< 0.0001
Be	0.458808	0.0945
B	0.984695	< 0.0001
F	0.891627	< 0.0001
Si	0.897715	< 0.0001
S	0.901255	< 0.0001
Ti	0.583277	0.0024
Cr	0.778875	0.0007
Mn	0.97467	< 0.0001
Fe	0.633119	< 0.0001
Co	0.673706	< 0.0001
Ni	0.837119	< 0.0001
Cu	0.775084	< 0.0001
Zn	0.968975	< 0.0001
Ga	0.968258	< 0.0001
As	0.868749	< 0.0001
Se	0.752057	< 0.0001
Rb	0.93791	< 0.0001
Sr	0.956136	0.0003
Y	0.906379	< 0.0001
Zr	0.957105	0.612
Nb	0.700007	0.0134
Mo	0.976652	0.3755
Ag	0.27926	< 0.0001
Cd	0.564456	0.0318
Sn	0.343915	< 0.0001
Sb	0.877714	< 0.0001
Cs	0.366592	0.04
Ba	0.9714	0.0032
La	0.921852	0.0007
Ce	0.510285	< 0.0001
Pr	0.622795	0.0107
Nd	0.670928	0.0007
Eu	0.801993	< 0.0001
Tb	0.574121	0.0107
Ho	0.343968	0.3753
Tm	0.683318	0.0005
Ta	0.355203	0.3379
Hg	0.761677	< 0.0001
TI	0.91434	< 0.0001
Pb	0.940814	< 0.0001
Bi	0.751279	< 0.0001
Th	0.601846	< 0.0001
U	0.812672	< 0.0001

source. The statistical analysis employed in this study show that the differences are statistically significant. The means of all these elements in the different regions, accompanied by the above-mentioned statistics that describe their consistency and reliability of estimate, may now be saved in a data-base which would allow well founded comparisons with future or past estimates which, hopefully, will also have been, or will be, properly analysed. Furthermore, possibly interesting information regarding the process of laying down of dentine and/or in the application of the chemo analytic techniques, may be revealed by closer investigation of those elements that exhibit the greater error of variance, that is large P values.

Fingerprinting ivory samples appears to demand chemical analyses of a highly sophisticated order to provide the range of elements and measurement accuracy that is needed for successful classification. However, these data are of little value without the as highly sophisticated and extremely flexible statistical analytic software which is available in the modules of the SAS system. Only when these two sciences are brought together can an integration of information be achieved which results in real knowledge. It is this knowledge that may play an important role in the conservation of the elephant herds of South Africa and may provide a model for the conservation of many other precious natural resources.

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REFERENCES

Barnes, R. F., C. G Craig., H. T. Dublin., G. Overton., W Simmons and C. R Thouless (1999) African elephant database 1998. Occasional paper of the IUCN species survival commission. No.2. IUCN, UNEP, Gland, Switzerland, Cambridge, UK

Blanc, J. J., C. R Thouless., J. A Hart., H. T Dublin., I Douglas-Hamilton., C. G Craig and R. F Barnes (2003). African elephant status report 2002. An update from the African elephant database. Occasional paper of the IUCN species survival commission No.29. IUCN, UNEP, Gland, Switzerland, Cambridge, UK

Dolphin, A., A. Goodman and D. Amarasiriwardena (2005) Variation in enamel intensities among teeth and between pre- and postnatal regions of enamel. Am J Phys Anthropol. 128, 878-888.

Lochner. F., J. Appleton., F. Keenan F and M. Cooke (1999) Multi-element profiling of human deciduous teeth by laser ablation-inductively coupled plasma-mass spectrometry. Anal Chim Acta. 401, 299-306

Posner. A. and P. Tannenbaum (1984). The mineral phase of dentine. In: Linde A, editor. Dentine and Dentinogenesis Florida: CRC Press, Boca Raton, p. 17-36.

Raubenheimer. E., J. Brown., D. Rama., M. Dreyer., P. Smith and J. Dauth (1998). Geographic variations in the composition of ivory of the African elephant. Arch Oral Biol. 43: 641-647

Van der Merwe, N. J and J. A Lee-Thorp (1990) Source-area determination of elephant ivory by isotopic analysis. Nature, 346: 744

Vogel, J. C., B. Eglington and J.M Auret (1990) Isotope fingerprints in elephant bone and ivory. Nature, 346:747.