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Trace element analysis of bighorn sheep horns

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Trace element analysis of bighorn sheep horns

Weigel, Peter Gordon, M.S.

University of Nevada, Las Vegas, 1990

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**TRACE ELEMENT ANALYSIS OF
BIGHORN SHEEP HORNS**

by

Peter G. Weigel

**A thesis submitted in partial fulfillment
of the requirements for the degree of**

Master of Science

in

Biology

**Department of Biological Sciences
University of Nevada, Las Vegas**

February 1990

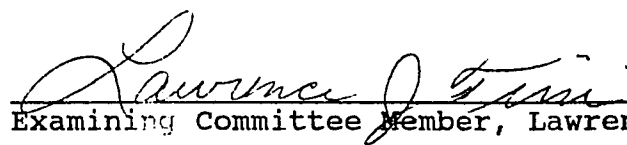
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University of Nevada, Las Vegas
February 1990

ABSTRACT

A total of 249 samples of horn material from bighorn sheep was collected from nine locations. The concentrations of eight elements were determined in all samples by inductively coupled argon plasma spectroscopy. Year of horn growth was determined for 154 of the samples. All element concentration varied significantly with respect to location. Some elements varied similarly to other elements with respect to location, but most locations exhibited unique element concentration profiles. Six of eight elements exhibited significant variation with respect to year of horn growth. A general pattern of annual element concentration fluctuation appeared to emerge. Generally, for a given year, if one element occurred in relatively higher or lower concentrations with respect to the previous year, the other study elements were found to occur in relatively higher or lower concentrations, respectively, as well. Neither plant element concentrations nor precipitation were found to be significantly related to horn element concentrations. Correlation analysis of bighorn sheep hair element concentration and horn element concentration revealed no significant relationship.

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INTRODUCTION

Mineral analyses of many kinds of biological sample materials have provided researchers in a wide variety of disciplines useful information and unique insights into otherwise untapped areas. Depending upon the average concentration and frequency of which different minerals are found in animals, they have been generally classified into two groups: those that form the bulk of living matter, approximately 99.9% (H, C, N, O, Na, Mg, P, S, Cl, K, and Ca), and those that are considered essential trace elements, the remaining 0.1% (F, Si, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, Sn, and I) (Valkovic 1977, Dormandy 1986).

Much work has been done concerning specific biological roles that different minerals play in various systems (Nicholas and Egan 1975, Underwood 1977, Peereboom 1985). Some studies have focused on the use of mineral profiles found in various sample materials in answering questions pertaining to the location or origin of organisms from which samples have been collected (Picton and Eustace 1986, Bortolototti et al. 1988, Bortolototti et al. 1989). Other studies are concerned simply with the accuracy in which mineral concentrations

are measured, and the validity of using a particular tissue for the purpose of elemental analysis (Hopps 1977, Maugh 1978, Errera 1980, Holzbrecher and Ryan 1982, Monsaterios et al. 1986). In relatively recent years, with the development of more sensitive detection equipment, researchers in the human health field have greatly explored the use of human hair as a biopsy material for studying both nutritional aspects and environmental effects of trace element contents (Gibson and DeWolfe 1979, Katz 1979, Gordon 1985, Limic and Valkovic 1986, Lal et al. 1987). The attraction of the use of hair comes from the ease of sample collection and storage, the stability of element content within the hair, and the fact that hair acts as a recording filament which can reflect varying levels of trace elements in the body over a long period of time (Harrison et al. 1969, Errera 1980, Monasterios et al. 1986).

Being made of keratin, hair is probably one of the most stable structures synthesized by animals with respect to chemical composition. It is the presence of relatively high concentrations of cystine molecules (the resultant disulfide bound combination of two cysteine amino acid molecules) which provide the great strength and stability found in keratin based tissues (Valkovic 1977). According to Valkovic (1977), X-ray studies have established that hair, nails, hooves, and horn are

all similarly constructed; all are made of keratins, and all show high concentrations of cystine as compared with other amino acids.

The inert condition of keratin based materials, once they are synthesized, provides for their attractive application for studies of trace elements in animals. While keratins are being synthesized, trace minerals are deposited in concentrations which can reflect the current metabolic status of the animal, and these elements are bound up in the closely knit mass of polypeptide chains comprising the structure of the tissue being produced (Valkovic 1977, Monasterios et al. 1986). Once locked into the keratin matrix, very little change in trace element concentration can occur. Some studies on human hair have revealed that external environmental factors may be affecting trace element concentrations due to adsorption onto the hair surface and diffusion into the immediate outer portion of the cortex (Hopps 1977, Roberts and Green 1985, Limic and Valkovic 1986). Other keratin based materials such as nail are not as susceptible to surface contamination due to a much smaller surface area to mass ratio. Regardless of the material being analyzed, most authors recommend a vigorous pre-analysis washing procedure (Hambidge et al. 1972, Assarian and Oberleas 1977, McKenzi 1978).

Trace element studies outside the human health

field have largely focused on the health relations to livestock, some anthropological questions, and a few applications involving wildlife investigations. Much work on livestock and trace elements in soil and forage was reviewed by Nicholas and Egan (1975).

Anthropological studies have included diet reconstruction of early North American peoples (Byrne and Parris 1987), and lead concentrations in ancient Peruvians as compared with present day people (Ericson et al. 1979). Bird feathers, which are also constructed of keratin proteins, have been used in situations analogous with human hair. Bartolotti et al. (1988, 1989) have shown both spatial and temporal variation in trace element concentrations of grouse feathers. They were also able to relate trace element content to differences in grouse habitat. Picton and Eustace (1986) used trace element profiles from drill shavings from bighorn sheep horns to produce a binary key for use in identifying the general area of origin of sampled sheep. This study initially attempted to pinpoint more specific locational groupings, but the element profiles were not distinct enough to provide for such fine separations.

Bighorn sheep (Ovis canadensis) are widely recognized by the large curled horns possessed by rams. Geist (1965, 1966a) has provided evidence supporting the hypothesis that ram horns evolved as weapons for

intraspecific combat, and presently function primarily as display organs and dominance indicators. He supports this view with evidence that "large horned rams enjoy a reproductive advantage". Bighorn sheep horns grow incrementally and produce annual growth rings (segments) which can be used to accurately determine the age of individual sheep (Geist 1966b). Relative horn growth in Dall sheep (Ovis dalli) has been shown to be a function of precipitation, an indicator of habitat quality, and correlated with recruitment (Bunnell 1978). Wehausen and Douglas (1982) examined horn sheaths showing deficient growth from rams that died due to a pneumonia epidemic at Lava Beds National Monument in 1980 and suggested that the horns "indicated substantial nutritional or other stress during the (previous) growing seasons that led to low allocation of nutrients" to the animals.

This study focuses on the measurement and description of both spatial and temporal variation of trace element concentrations in horns from bighorn sheep. The role of plant element content and precipitation influences on horn element content is investigated, and the relationship with bighorn sheep hair element content is also explored.

METHODS

Sample Collection

Horn samples from bighorn sheep were collected from animals inhabiting a variety of areas, including Death Valley, California; southern Nevada; and western Arizona. A total of 249 horn samples was collected and divided into nine groups based upon geographic similarities. The nine groups and their sample sizes are as follows:

1. Death Valley National Monument, California (Cottonwood Mts., Black Mts., Funeral Mts., and Grapevine Mts.) Sample size = 24.
2. Esmeralda County, Nevada (Stonewall Range, Silverpeak Mt., and Lone Mt.) Sample size = 7.
3. Sheep Mt. Range, Nevada. Sample size = 16.
4. Las Vegas, Nevada area (Arrow Canyon, Las Vegas, Meadow Valley, and Mormon Mt.) Sample size = 13.
5. Desert Range, Nevada (Desert Mts., and Pintwater Range) Sample size = 12.
6. McCullough Range, Nevada (Black Mts., Muddy Mts., and Eldorado Mts.) Sample size = 33.
7. Lake Mead National Recreation Area, Nevada and Arizona (Little Gyps Cove NV, Boulder Wash NV,

Coyote Cove NV, River Mts. NV, Sidewinder Cove AZ, Indian Canyon AZ, Fish Finder Cove AZ, and Willow Beach AZ) Sample size = 107.

8. Lower Colorado River, Arizona. Sample size = 24.

9. Southwest Arizona. Sample size = 13.

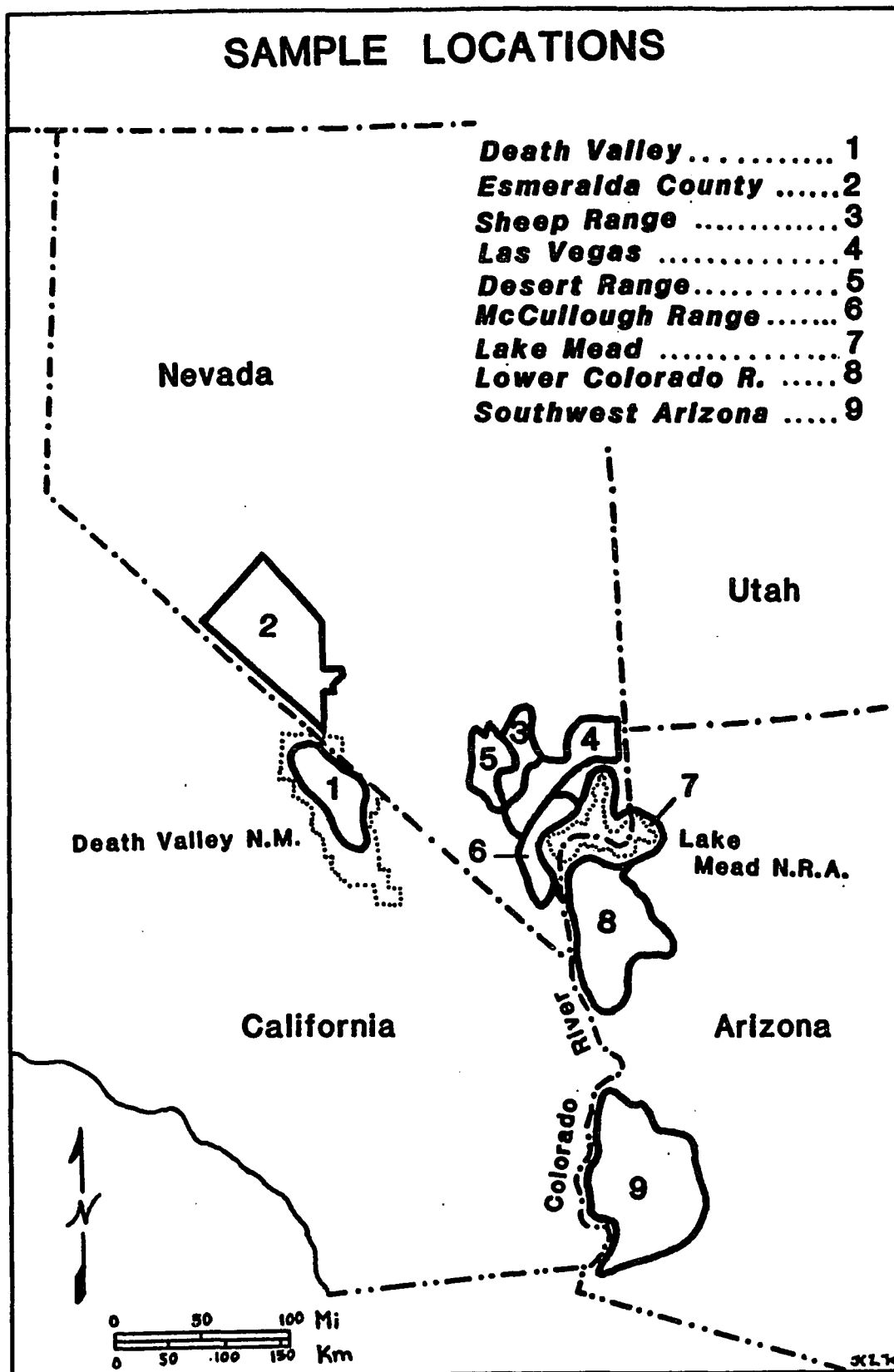
Figure 1 shows a map indicating the location of the nine sample groups. Considering the topography and average mobility of bighorn sheep, (Simmons, 1980) it was felt that the groupings represented geographically distinct populations, although some overlap may be present.

Horn samples were collected using three separate methods. These methods varied between groups, however, no single group was sampled using more than one method.

Horn Samples from group 1 were collected from skulls of bighorn sheep rams housed in the museum collection at Death Valley National Monument. With the aid of catalog information and the museum curator, the location of original discovery of the skulls was determined. All skulls had been found in or near one of the four mountain ranges mentioned previously in the grouping information. Samples were taken using a 3/8 inch variable speed drill and a 1 inch diameter wood drill bit. Horn material was removed from the skull by drilling into the horn sheaths. Initially, the surface layer (approximately 1 mm) was removed and discarded in order to reduce the risk of contamination due to soil and other surface debris. The sample was

Figure 1. Map of the nine bighorn sheep horn sample acquisition locations.

SAMPLE LOCATIONS



then taken by continuing to drill into the same hole another 2-3 mm. This method provided a sample of 1-2 grams.

Horn samples comprising group 7, the Lake Mead National Recreation Area, were collected from live animals captured during live trapping operations. The trapping was conducted as part of a relocation effort on the part of the Nevada Department of Wildlife and Arizona Department of Game and Fish. Sampling was conducted with the permission and help of those agencies. Bighorn sheep were trapped by using a 455 m² drop net while they were feeding underneath. Once captured, the sheep were blindfolded and hobbled, and the respective state agency would then note pertinent information including age, sex, location, general condition, etc., while the horn samples were being taken. Samples needed to be taken quickly and harmlessly, and this precluded the use of the drill method described earlier. Instead, a small portion (1-2 grams) of the animal's horn was removed by nipping the tip off with farrier's pliers, or horse hoof nippers. The sample was placed in a small envelope which was marked with an identification number given to the particular animal by the State agency. This number was later used to access information pertaining to each animal from which samples were taken. Of particular interest was the animal's age. By knowing the year that

the sample was taken and the animal's age it was possible to determine the year in which the horn was produced from which the sample came. This information was quite pertinent to some aspects of this study. Because of the ease of access and acquisition, hair samples were also taken from animals during the trapping operations around Lake Mead. Hair was collected by plucking looser clumps and simply brushing the animal with a hand. Any loose hairs were gathered and deposited in an envelope marked with the animal's identification number. Sample weights of hair ranged from 0.1 g - 1.5 g. Hair samples were only collected from animals belonging to group 7.

All other horn samples were collected by either the Nevada Department of Wildlife (Groups 2-6) or the Arizona Department of Game and Fish (Groups 8 and 9). These horn samples were taken from bighorn rams harvested during the 1987 hunting seasons. According to the states' game laws, hunters are required to present their quarry to the respective state agency for inspection and validation. In order to insure future identification of the mounted head, a small metal plug containing an identification number is inserted into a small hole drilled into the ram's horn. The two state agencies use similar methods in mounting the metal identification plug. Of interest to this study is the fact that a hole approximately 0.5 inch deep and 3/8

inch in diameter was drilled into the horn material. The two state agencies were kind enough to save the drill shavings for use in this study. Along with the horn sample, the agencies provided information on the animal's location, age, and from which annual growth ring of the horn the sample was taken. Horns of bighorn sheep grow incrementally with well defined annual growth rings. By counting back from the skull to a given growth ring, one can determine the year in which the material from that ring was produced (Geist, 1966).

Sample Preparation and Elemental Analysis

Sample preparation procedures were adapted from recommendations by the International Atomic Energy Agency as presented in Monasterios et al. (1986) and Clanet et al. (1982). Horn samples in the form of drill shavings were washed twice with 20 ml of acetone in a clean 250 ml beaker. Shavings were agitated for approximately 1 minute in each application of acetone. The used acetone was discarded. The horn shavings were then transferred to a Buchner funnel and rinsed three times with 50 ml of deionized water. The samples were then transferred to aluminum drying pans and placed in a drying oven at 60° C for 24 hours. Once dried, the samples were weighed on an analytical balance to the nearest 0.001 g. If sample size permitted, 0.5 g was weighed out for analysis and the remainder was

discarded. If the sample was smaller than 0.5 g, the entire portion was used. Once weighed, the horn material was placed into acid washed (with concentrated HNO₃) 100 ml Pyrex beakers. Ten ml of concentrated HNO₃ was added to each beaker which was then covered with Parafilm and allowed to stand at room temperature for 12 hours. This step began the digestion process. To prevent contamination, only Ultrex HNO₃ for analytical work was used for both washing of glassware and use in digestion and dilution. Following initial digestion, the beakers and their contents were heated on electric hot plates. The sample solutions were boiled until nearly all liquid had evaporated, at which point another 10 ml of concentrated HNO₃ was added to each beaker. This solution was also boiled down, leaving the horn material fully digested. At this point 30 ml of a 0.03 M HNO₃ solution was added to the solution in order to dilute the concentrated acid. This solution was boiled down to approximately 5 ml, and another 30 ml of 0.03 M HNO₃ was added. This solution was boiled down to below 20 ml and allowed to cool. The final cool solution was transferred to acid washed 25 ml volumetric flasks, and brought up to volume (25ml) with 0.03 M HNO₃. The final horn concentrations of the solutions were calculated; most samples were 0.5 g in 25 ml or a 1:50 dilution. This figure was required for determining final elemental concentrations of each

sample.

Horn samples acquired using the farrier's pliers (group 7 from the Lake Mead area) were prepared exactly as the drill shaving samples with the addition of the following preliminary steps. The horn tips were first scrubbed with a tooth brush under running deionized water, and then cut into small (approximately 25 mm²) pieces. The scrubbing removed surface debris not found in the drill shavings, and cutting into small pieces accelerated the acid digestion process.

Hair samples were also prepared according to the procedures outlined above for horn shavings, but they often required more than two acetone washes. Hair samples were washed with acetone up to four times, or until the discarded acetone appeared clear and showed no signs of contamination from dirt or dust. Hair sample weights were recorded to the nearest .001 g for use in calculating final concentrations.

All samples were analyzed using a Perkin-Elmer Plasma 40 inductively coupled argon plasma spectrometer (ICAPS). This atomic emission spectrometer was able to perform multiple element analyses of a given sample, and provide a printed report detailing the concentration of all elements in question. Initially, samples were to be analyzed for 19 separate elements. Given time restraints and the fact that many of these elements were undetectable in trial run samples, the number of

elements analyzed was decreased to eight. Sample analysis elements included: Zinc (Zn), Phosphorus (P), Iron (Fe), Magnesium (Mg), Copper (Cu), Calcium (Ca), Sodium (Na), and Potassium (K). A list of these elements, the emission wavelengths used for detection, and the detection limits are provided in Table 1.

Two replicate runs were performed on each sample, and the mean value for each element was used in later statistical analyses. Sample analysis procedures were adapted from U.S. Environmental Protection Agency guidelines (USEPA Office of Solid Waste and Emergency Response, 1986). The ICAPS was calibrated prior to running any samples by first running high and low concentration standard solutions and a blank solution. These solutions were prepared by diluting 1000 ppm commercial standards with 0.03 M HNO₃ (Ultrex). The blank consisted of only 0.03 M HNO₃. All solutions were prepared using disposable pipets and acid washed glassware. Once calibrated, the ICAPS was checked for accuracy before each run by running an initial calibration verification (ICV) solution. This solution was obtained from the Quality Assurance Laboratory (QAL), Environmental Research Center, University of Nevada Las Vegas. The QAL is required to use ICV solutions when performing work contracted by the U. S. Environmental Protection Agency (EPA). Following EPA guidelines, if the ICAPS calibration was more than ten

Table 1. List of analysis elements with corresponding emission wavelength (nm) used for detection, and lower detection limit (ppm).

<u>ELEMENT</u>	<u>WAVELENGTH (nm)</u>	<u>DET. LIMIT (ppm)</u>
Zn	213.856	0.0016
P	214.914	0.076
Fe	259.940	0.0062
Mg	279.553	0.00015
Cu	324.754	0.0054
Ca	422.673	0.010
Na	589.592	0.069
K	766.490	0.021

percent off when the ICV was run, then the standards and blank were run again and the new calibration was rechecked. Samples were analyzed only when ICV concentration readings were within ten percent of their true values. The ICV solution was run periodically (every 5-10 sample runs) to ensure that the ICAPS calibration remained within acceptable boundaries. If ICV readings were more than ten percent off, standard solutions were rerun and the instrument was recalibrated.

RESULTS

Geographic Variation

One way analysis of variance tests were performed on each element with respect to location. These eight tests revealed concentrations of all individual elements to vary significantly with respect to location of sample acquisition. Table 2 lists F-statistic and p-value results of ANOVA for individual elements with respect to location. There were highly significant differences between locations ($p < .0001$) for all elements. Table 3 lists mean concentrations and 95% confidence intervals for each element, and Figures 2-9 present mean value and 95% CI plots for each element. Average concentrations of elements ranged widely from values near 2 ppm for copper to values as high as 1769 ppm for potassium. Due to the large range of concentrations, all eight elements could not be compared simultaneously, because plotting them together would tend to dampen subtle fluctuations seen in elements occurring at lower concentrations. Therefore, the elements were divided into two groups of four. The first group consists of elements which occur at lower concentrations (2-250 ppm) and includes Zn, Fe, Mg, and Cu (see Figure 10). The higher concentration group (200-1770 ppm) includes P, Na, Ca, and K (see

Table 2. F-statistic and p-value results of ANOVA for the eight analysis elements with respect to location group.

<u>ELEMENT</u>	<u>F-ratio</u>	<u>p-value</u>	<u>d.f. total</u>
Zn	7.077	<.0001	248
P	52.458	<.0001	248
Fe	32.965	<.0001	248
Mg	40.021	<.0001	248
Cu	31.427	<.0001	248
Ca	14.225	<.0001	248
Na	16.149	<.0001	248
K	97.540	<.0001	248

Table 3. Mean concentration values (ppm) and 95% C.I. for the eight study elements with respect to location group of sample origin.

ELEMENT	LOCATION GROUP NUMBER			
	1	2	3	4
Zn	129.6±6.9	141.9±13.0	118.1±8.6	126.0±9.5
P	437.5±68.1	598.6±126.1	524.4±83.5	443.1±92.5
Fe	50.5±9.6	6.2±17.9	4.8±11.8	7.6±13.1
Mg	211.3±19.9	160.8±36.8	140.0±24.4	149.2±26.9
Cu	6.7±0.6	4.8±1.2	1.7±0.8	1.8±0.9
Ca	745.9±65.9	568.6±121.9	492.5±80.7	504.0±89.5
Na	385.5±134.4	811.6±248.6	833.5±164.5	887.6±182.4
K	195.4±150.7	502.8±279.0	490.8±184.6	439.3±204.7
	n=24	n=7	n=16	n=13

ELEMENT	LOCATION GROUP NUMBER			
	5	6	7	8
Zn	103.0±9.9	121.6±6.0	111.7±3.3	119.4±7.0
P	426.1±96.3	462.2±58.0	900.9±32.3	457.0±68.1
Fe	5.0±13.7	14.0±8.3	61.6±4.5	11.5±9.7
Mg	130.0±28.0	147.9±16.9	260.5±9.4	150.7±19.8
Cu	5.9±0.9	4.0±0.6	3.0±0.3	6.6±0.7
Ca	467.4±93.2	483.7±56.2	712.4±31.2	522.8±65.9
Na	703.8±189.9	945.5±114.5	1064.9±63.6	658.5±134.4
K	450.3±213.0	556.6±128.4	1768.8±71.3	533.7±150.7
	n=12	n=33	n=107	n=24

Table 3. Continued.

ELEMENT	LOCATION GROUP NUMBER
	9
Zn	105.6±9.5
P	413.3±92.5
Fe	21.8±13.2
Mg	123.9±26.9
Cu	6.2±1.0
Ca	497.8±89.5
Na	381.9±182.4
K	352.1±204.7
	n=13

Figure 2. Mean concentration and 95% confidence interval bars for horn zinc for the nine sampling locations.

Figure 3. Mean concentration and 95% confidence interval bars for horn phosphorus for the nine sampling locations.

Figure 4. Mean concentration and 95% confidence interval bars for horn iron for the nine sampling locations.

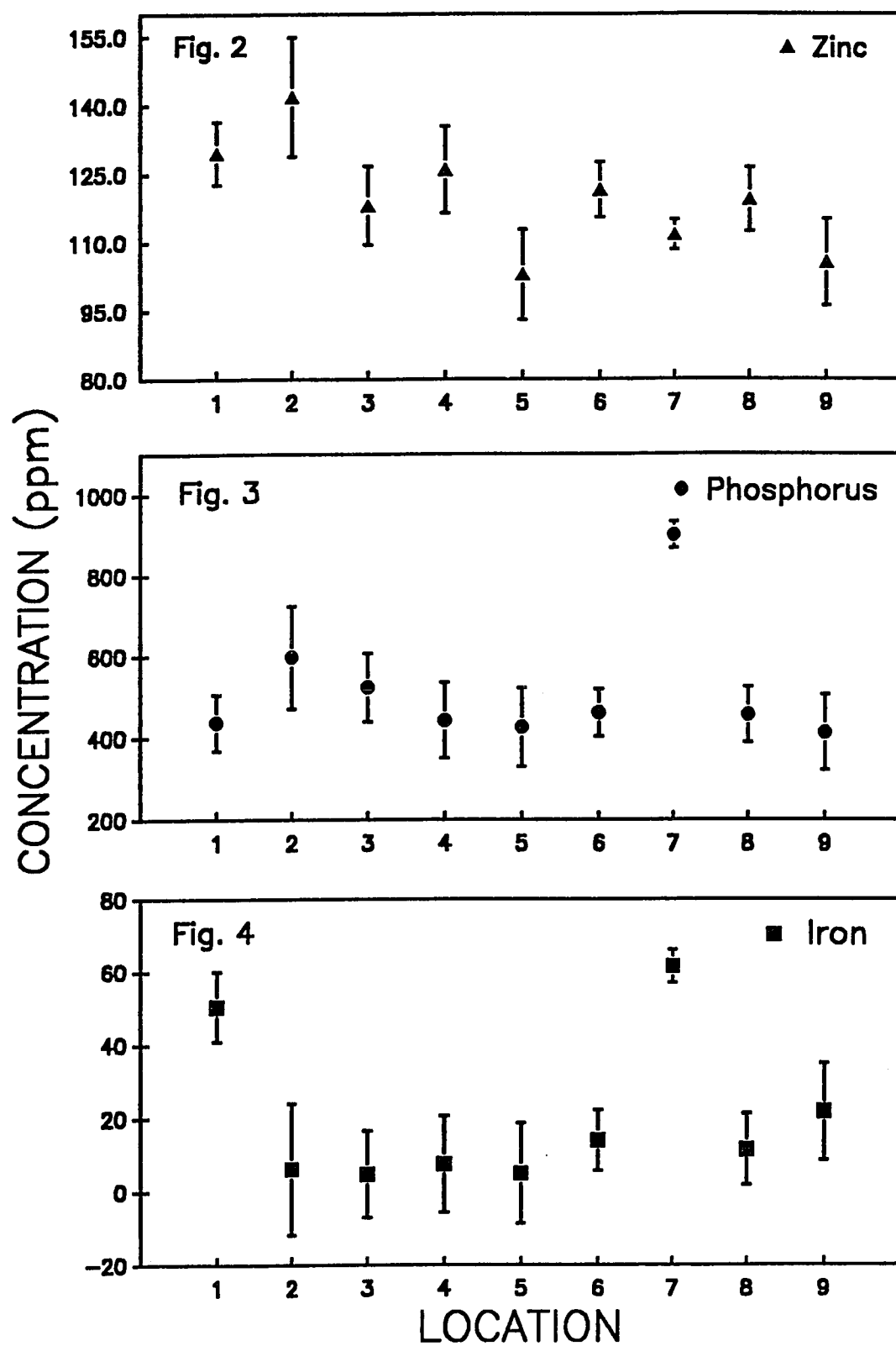


Figure 5. Mean concentration and 95% confidence interval bars for horn magnesium for the nine sampling locations.

Figure 6. Mean concentration and 95% confidence interval bars for horn copper for the nine sampling locations.

Figure 7. Mean concentration and 95% confidence interval bars for horn calcium for the nine sampling locations.

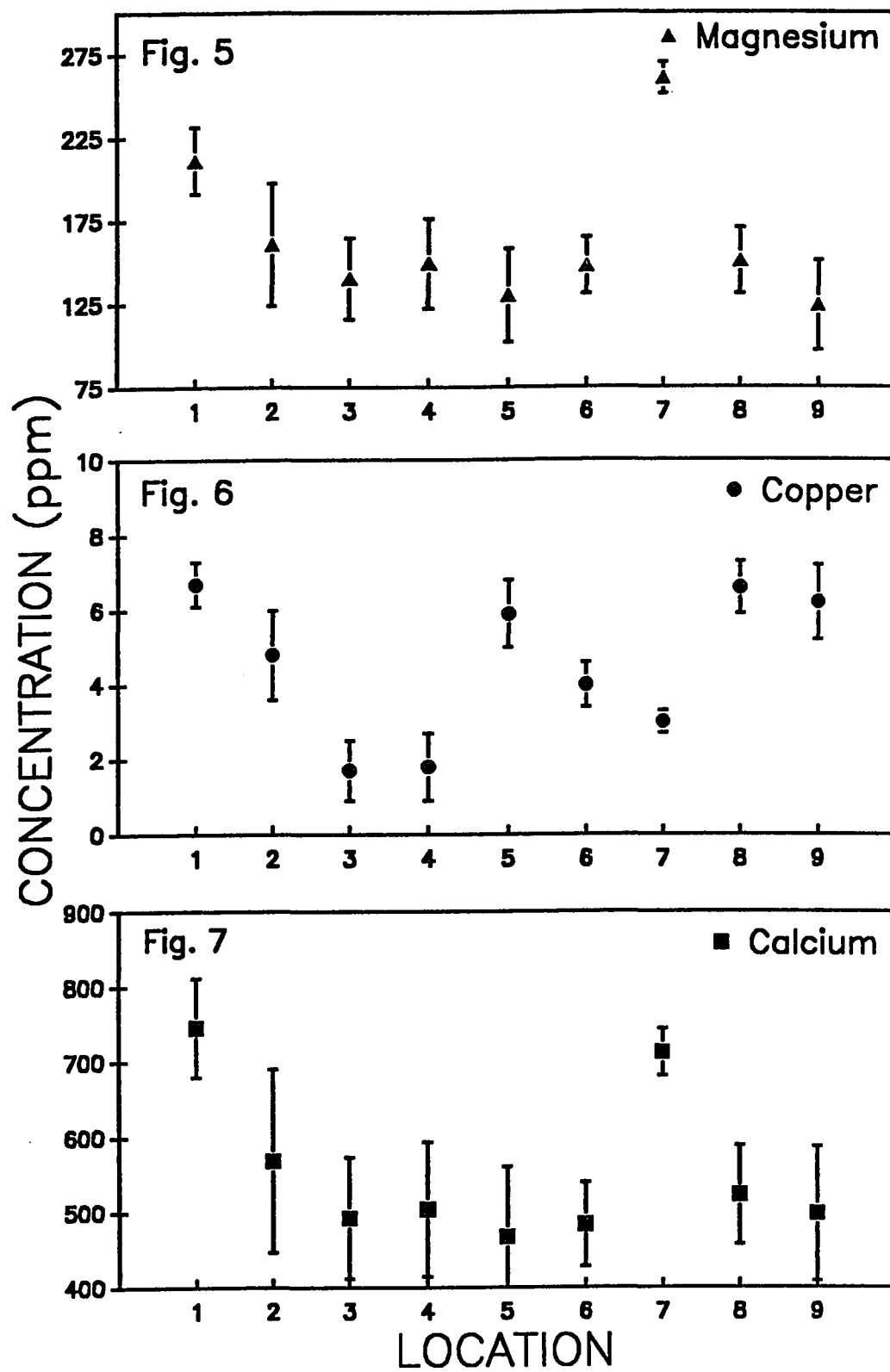


Figure 8. Mean concentration and 95% confidence interval bars for horn sodium for the nine sampling locations.

Figure 9. Mean concentration and 95% confidence interval bars for horn potassium for the nine sampling locations.

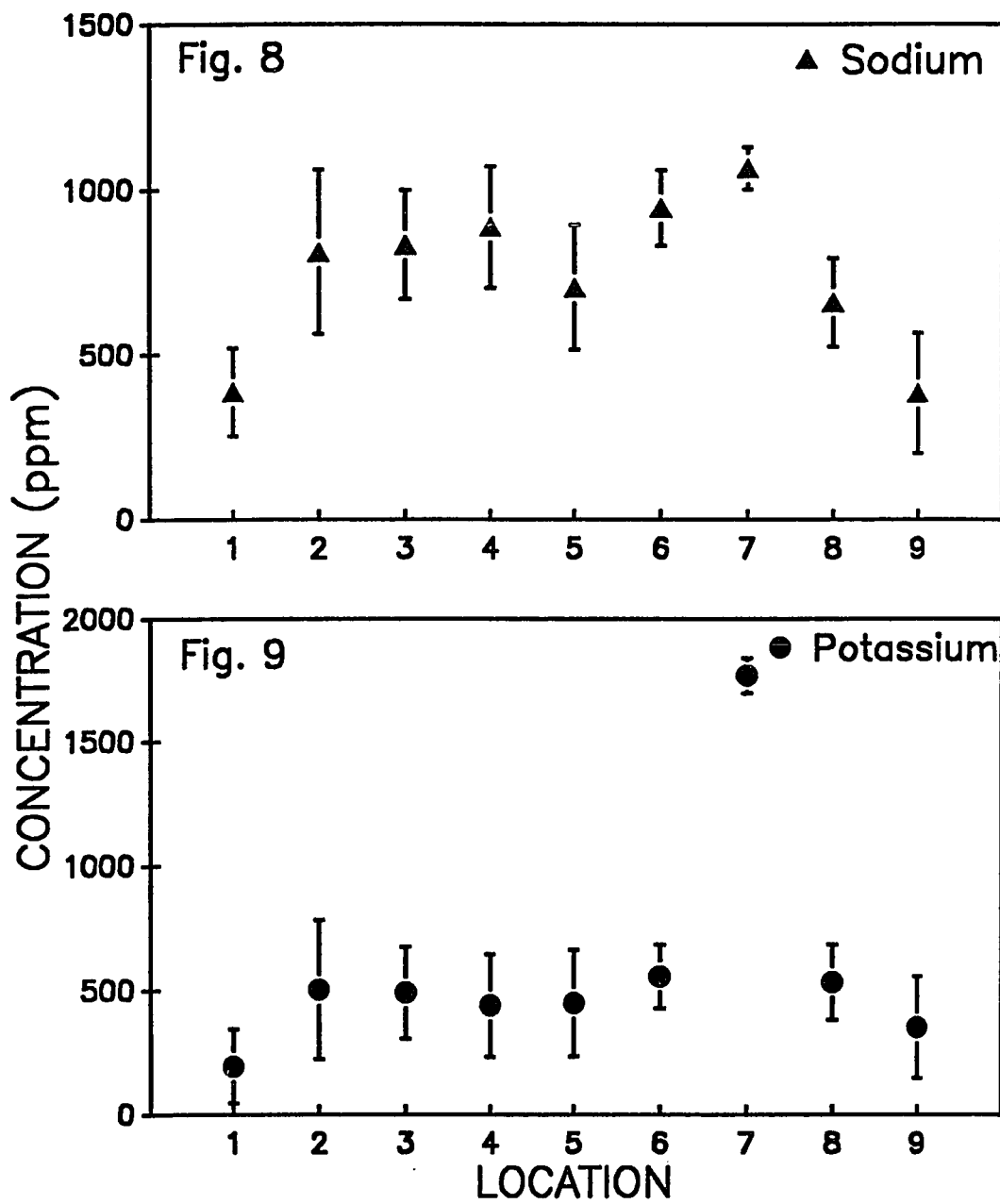


Figure 10. Mean concentration plots for horn zinc, copper, iron, and magnesium for the nine sampling locations.

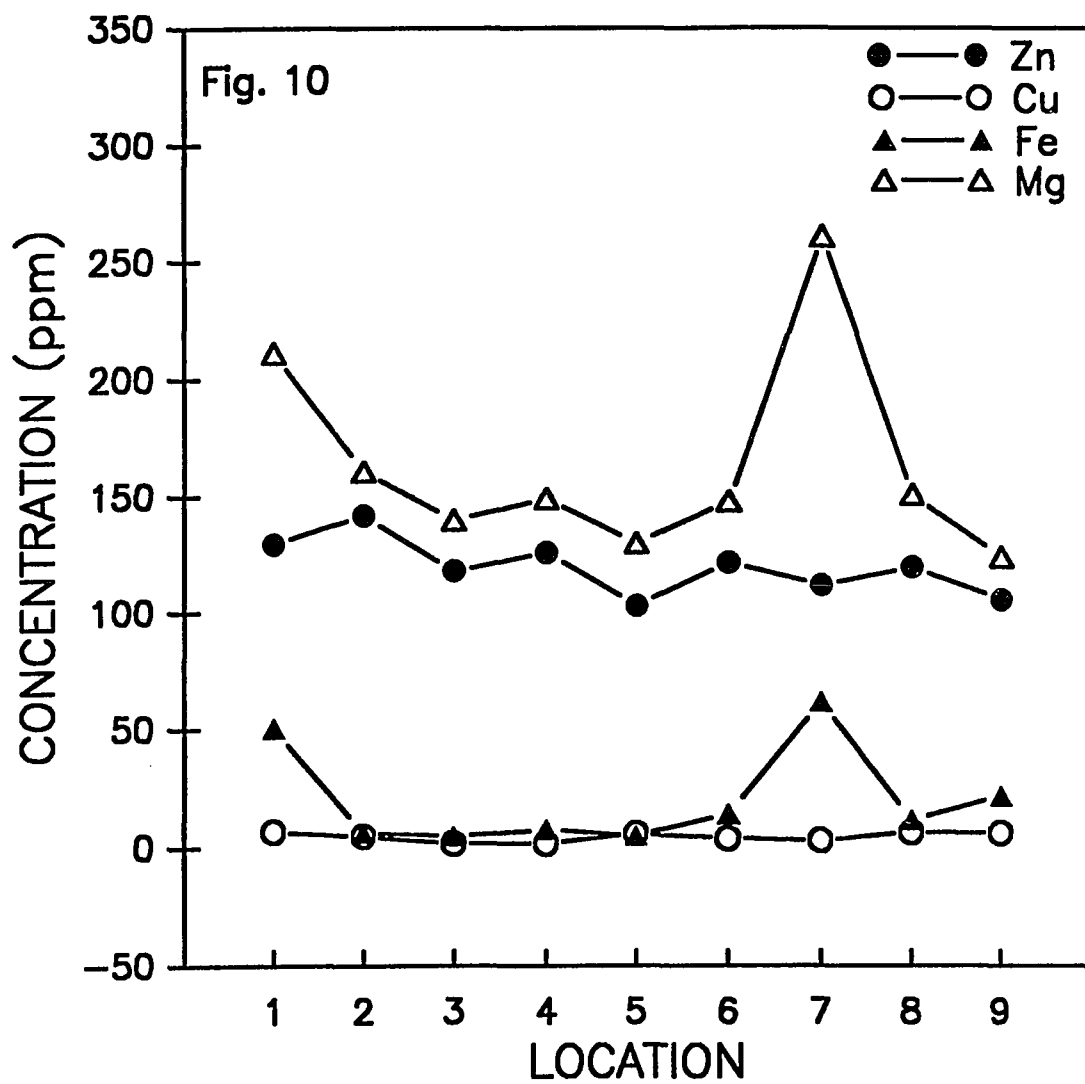


Figure 11. Mean concentration plots for horn
phosphorus, calcium, sodium, and potassium
for the nine sampling locations.

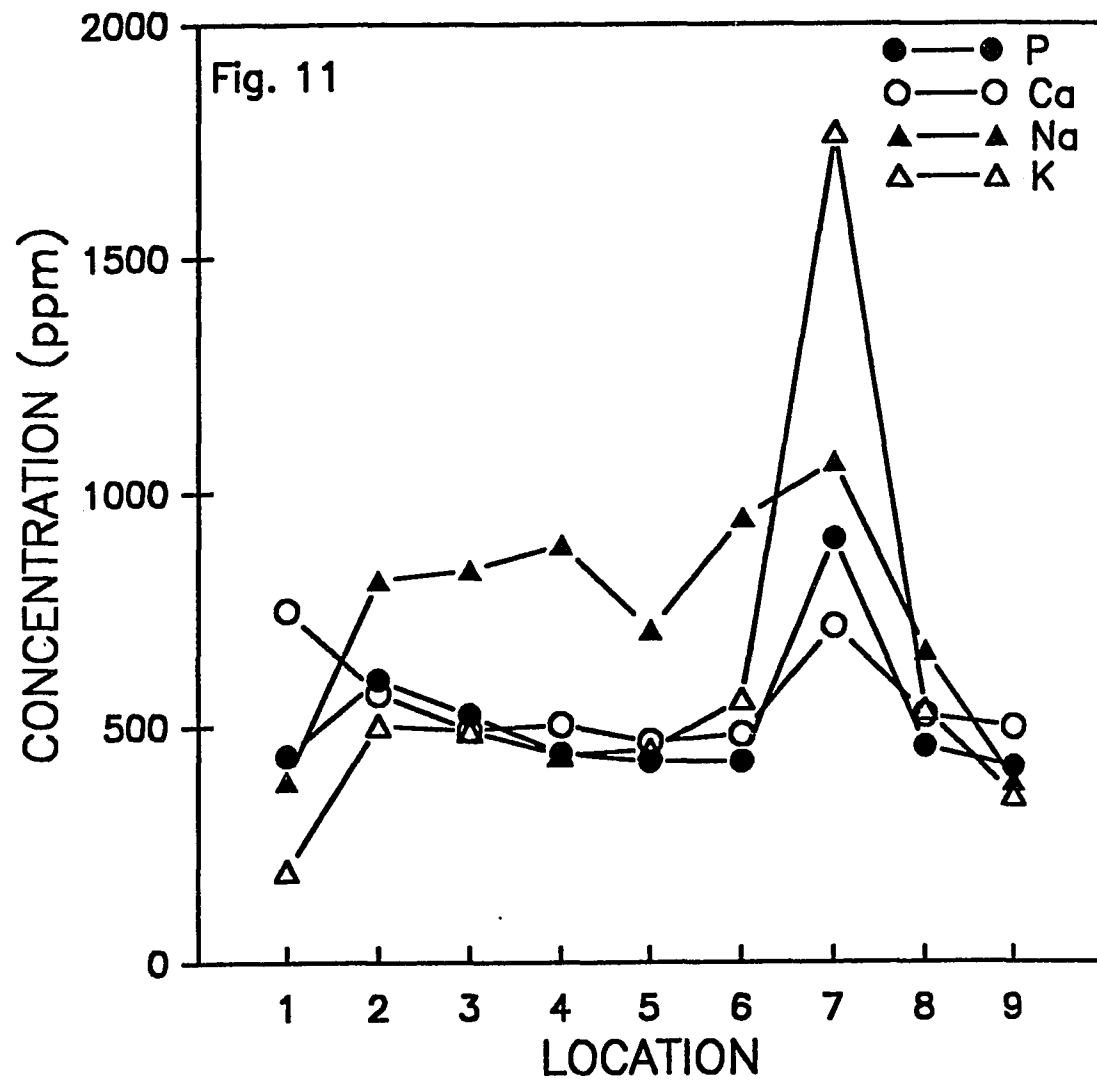


Figure 11). Once plotted together, most elements reveal similar concentration fluctuation patterns between locations. "Concentration pattern" is used here to describe the differences of concentration (ppm) that an element exhibits graphically between locations.

Although the concentration pattern for each element over the nine locations is relatively unique to that element, generally some portion of the pattern is repeated by several other elements as well. Most notable is the fact that, of the eight study elements, all except Zn and Cu exhibit a marked increase in concentration in horn samples taken from location 7, the Lake Mead area. Multiple range tests indicate that most elemental concentrations of samples taken from location 7 are significantly greater than elemental concentrations of samples taken from other locations ($p < .0001$).

Phosphorus, Mg, and K each show a significantly greater concentration at location 7 than any other location.

Iron concentration at location 7 is not significantly different from location 1, but locations 7 and 1 as a group shows significantly greater iron concentrations than the remaining seven locations taken as a group.

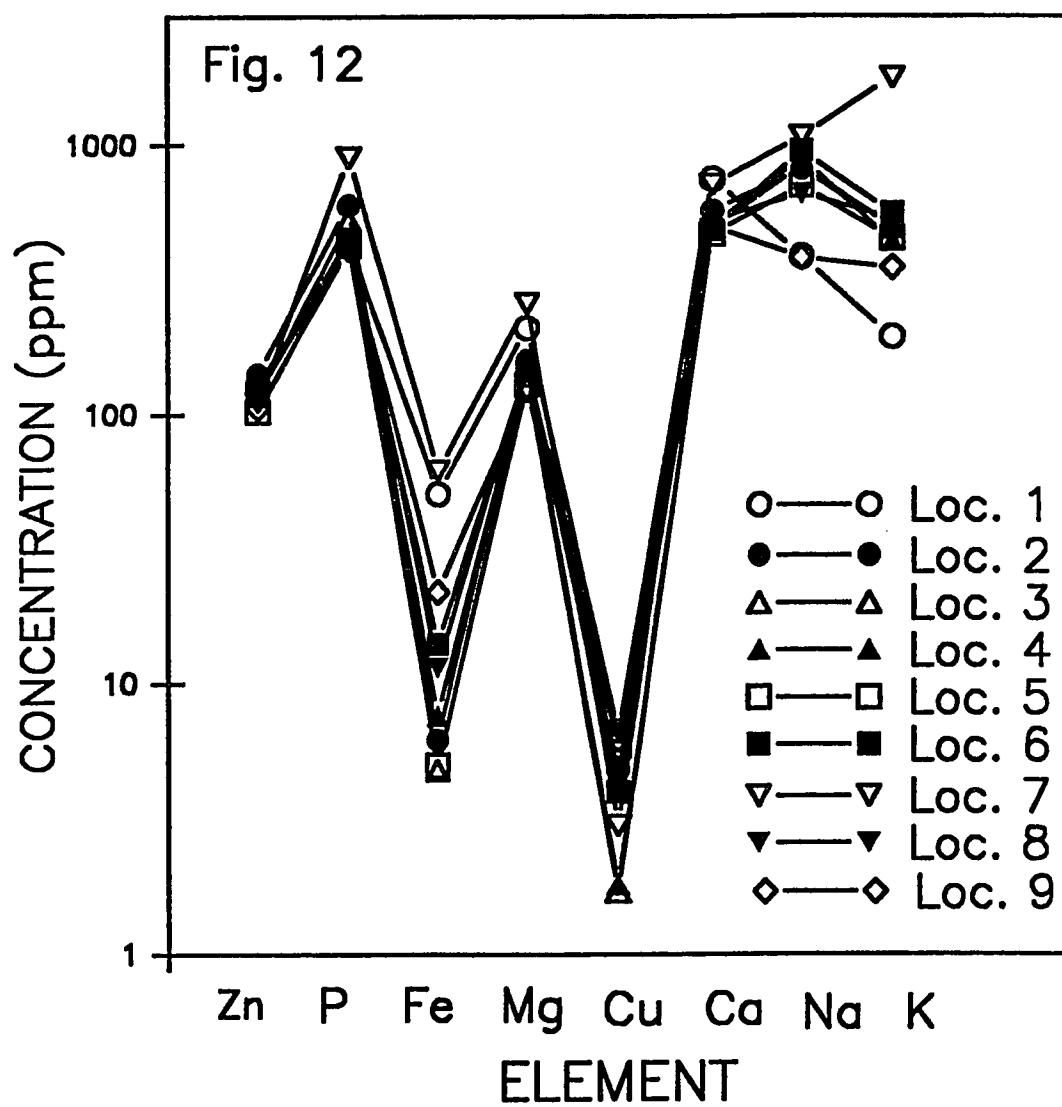
Calcium concentration at location 7 is not significantly different from locations 1 and 2, but, as was the case for iron, Ca concentration from locations 7 and 1 as a group was significantly greater than the remaining six locations (less location 2) taken as a group. Sodium

concentration at location 7 was not significantly greater from locations 2, 3, 4 ,or 6, but it was significantly greater than concentrations from locations 1, 5, 8, and 9. Also of interest is the strongly parallel high-low-high-low-high pattern of Zn and Mg between locations 2-3-4-5-6. Although not as prominent, this same pattern is also seen in Fe, Ca, and with the exception of location 2, Na as well. The only element that doesn't exhibit a recognizable pattern in Figures 10 or 11 is Cu. This is due to the relatively low concentrations in which it is found in sampled horn material. Figure 6, with its expanded scale, shows that Cu concentrations do fluctuate between locations, and although significant, this variance is considerably less than that of the other elements. Seven out of eight elements exhibiting moderately similar degrees of concentration variation between the nine different locations does seem important however.

In order to investigate the nature of the perceived concentration fluctuations further, the data were analyzed using the multivariate statistical technique of profile analysis. This procedure tests for parallelism among groups, where each group represents an independent set of population mean vectors (Johnson and Wichern, 1988). In the present case of elemental concentrations versus location, populations could not be defined according to element as has thus far been depicted. For

a given year of sampling, mean element concentrations were determined from a common group of samples. This fact violates the assumption of independent response populations. Prior to performing profile analysis, the data were transformed logarithmically, and transposed to produce groups based upon year of sample production. The log transformation was necessary in order to compensate for the wide range of values of individual element concentrations. Transposing the data allowed groups to be defined according to location, and this grouping represented independent populations, a necessary assumption for profile analysis. The locational groupings are depicted in Figure 12. Profile analysis here is testing for parallelism between the different location plots as a whole, and between separate pairs of locations. The different plots, do not necessarily need to coincide to be significantly similar, although if they do coincide, one can conclude that not only is elemental concentration fluctuation similar between locations, but also that the actual concentrations of elements is similar between locations. If groupings are found to be parallel, profile analysis performs the next test of coincidence. The results of profile analysis reveal that as a whole, the groups of locations do not exhibit significantly similar patterns of variation in elemental concentration ($p < .001$). When paired profile analysis tests were performed,

Figure 12. Log plot of mean elemental concentrations by locational group as used in profile analysis.



however, it was found that eight of 36 possible pairs of locations did exhibit significantly similar fluctuation patterns ($.080 < p < .427$). One other pair was nearly insignificant ($p = .047$), and with this additional pair taken into account, two groups of three locations each, and three separate pairs of locations could be regarded as not having similar element concentrations in sampled horn material, but at least exhibiting similar relationships between the concentrations of different elements. That is, the ratios of different element concentrations for particular locations were similar with those of some other locations, but not all other locations. Generally, this is what is observed in Figures 10 and 11. Some elements tend to vary similarly between some locations, while the variation is not so similar between other locations.

Temporal Variation

Concentrations of six of the eight study elements varied significantly (95% confidence level) from year to year when analysis of variance tests were performed. F-statistic and p-value results of ANOVA tests for individual elements are listed in Table 4. Zinc ($p=.9421$) and Ca ($p=.0564$) were the only two elements whose concentrations did not vary significantly with respect to year. As was the case with concentration ranges resulting from locational groupings, the eight

Table 4. F-statistic and p-value results of ANOVA for the eight analysis elements with respect to year of horn sample development.

<u>ELEMENT</u>	<u>F-ratio</u>	<u>p-value</u>	<u>d.f. total</u>
Zn	.355	.9421	153
P	12.994	<.0001	153
Fe	2.900	.0050	153
Mg	7.626	<.0001	153
Cu	2.513	.0138	153
Ca	1.953	.0564	153
Na	5.768	<.0001	153
K	10.700	<.0001	153

study elements, when grouped by year of horn growth, also exhibited a large range of concentrations (from a low of 2.9 ppm for Cu to a high of 2083.0 ppm for K). Table 5 lists mean concentration values \pm 95% C.I. for all eight elements with respect to year of horn sample growth. Because of the wide range of values the study elements were again grouped into two groups of four. The low concentration group of Zn, Fe, Mg, and Cu range in concentrations of 2.9-266.2 ppm, and their mean values are plotted simultaneously in Figure 13. The high concentration group of P, Ca, Na, and K range in concentrations of 414.8-2083.0 ppm and their mean values are plotted simultaneously in Figure 14.

Of the six elements that varied significantly from year to year, all except Cu exhibited almost identical fluctuation patterns. Copper concentrations were considerably lower than most of the other elements, and when graphed along with the others, appeared to vary only slightly. The five remaining highly significantly varying elements (P, Fe, Mg, Na, and K) all displayed concentration fluctuation patterns quite similar to one another. When analyzed on a year to year basis, this pattern becomes more apparent. The pattern appears as follows: (referring to Figures 13 and 14) concentrations dropped in 1980 with respect to 1979 levels (Na is an exception), they rose in 1981, dropped again in 1982, rose in 1983, remained fairly constant in 1984, and then

Table 5. Mean concentration values (ppm) and 95% C.I. for the eight study elements with respect to year of horn sample growth.

ELEMENT	MEAN \pm 95% C.I.			
	1979	1980	1981	1982
Zn	117.5 \pm 13.5	115.8 \pm 11.3	121.4 \pm 10.3	111.2 \pm 9.3
P	532.5 \pm 165.9	414.8 \pm 138.7	643.0 \pm 126.7	481.7 \pm 113.3
Fe	38.3 \pm 24.3	13.9 \pm 20.4	25.0 \pm 18.6	16.8 \pm 16.6
Mg	173.6 \pm 43.2	148.3 \pm 36.1	178.7 \pm 32.9	157.3 \pm 29.5
Cu	6.5 \pm 1.7	4.7 \pm 1.4	4.2 \pm 1.3	4.4 \pm 1.2
Ca	570.0 \pm 104.0	504.3 \pm 87.0	550.9 \pm 79.5	551.2 \pm 71.0
Na	631.6 \pm 227.1	682.9 \pm 190.1	843.7 \pm 173.5	632.3 \pm 155.1
K	727.0 \pm 469.2	458.0 \pm 392.6	926.5 \pm 358.4	560.6 \pm 320.7
	n=7	n=10	n=12	n=15

ELEMENT	1983	1984	1985	1986
Zn	115.2 \pm 9.9	115.4 \pm 7.3	114.4 \pm 5.6	112.1 \pm 8.7
P	580.6 \pm 121.7	562.8 \pm 89.6	748.7 \pm 69.4	889.0 \pm 106.4
Fe	27.6 \pm 17.8	30.0 \pm 13.1	35.5 \pm 10.2	40.3 \pm 15.6
Mg	179.5 \pm 31.7	178.0 \pm 23.4	212.1 \pm 18.1	252.3 \pm 27.7
Cu	3.9 \pm 1.2	3.5 \pm 0.9	3.2 \pm 0.7	2.9 \pm 1.1
Ca	580.1 \pm 76.3	589.1 \pm 56.2	606.8 \pm 43.5	661.2 \pm 66.8
Na	948.0 \pm 166.6	942.0 \pm 122.6	1058.6 \pm 95.0	1111.6 \pm 145.7
K	837.5 \pm 344.3	984.3 \pm 253.4	1332.4 \pm 196.2	1623.6 \pm 301.0
	n=13	n=24	n=40	n=17

Table 5. Continued

<u>ELEMENT 1987</u>	
Zn	115.4±8.9
P	1057.4±109.7
Fe	62.9±16.1
Mg	266.2±28.6
Cu	3.2±1.1
Ca	665.9±68.8
Na	1085.6±150.2
K	2083.0±103.1
n=16	

Figure 13. Mean concentration plots for zinc, iron
magnesium, and copper by year of horn
sample production for all locations.

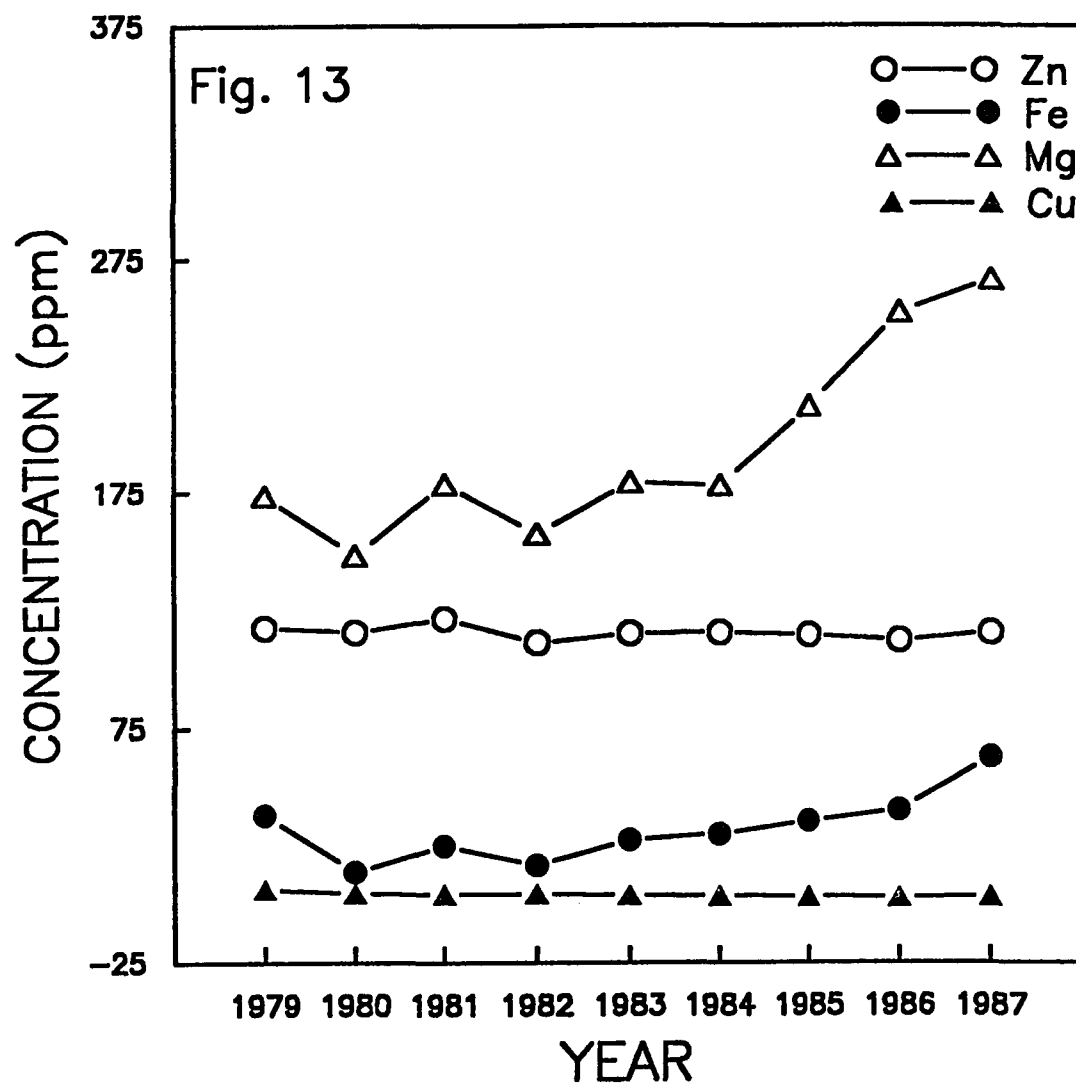
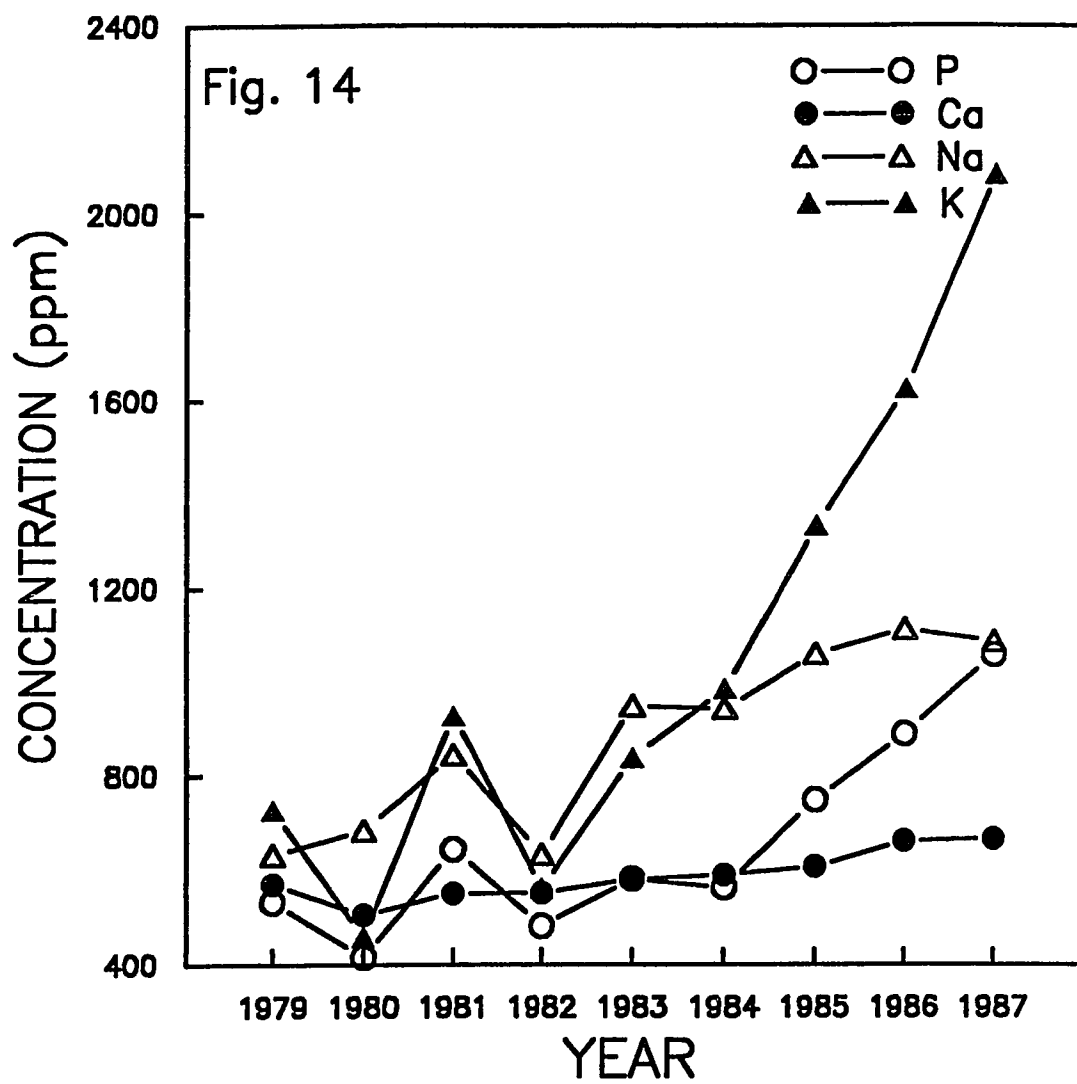


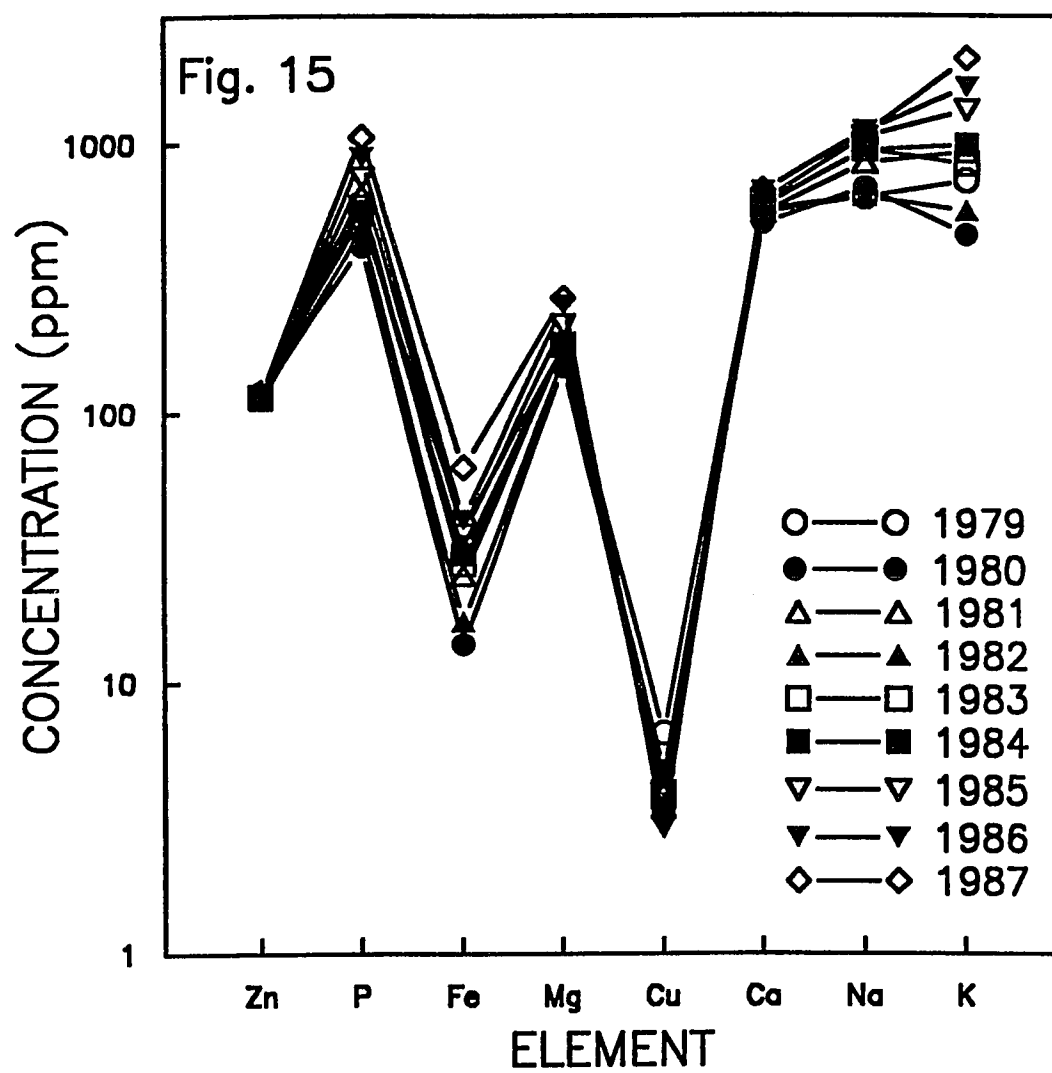
Figure 14. Mean concentration plots for phosphorus, calcium, sodium, and potassium by year of horn sample production for all locations.



rose continually from 1985 thru 1987 (Na again was an exception when concentrations remained fairly constant from 1986-1987). Zinc, although not exhibiting significant variation, did follow the same pattern somewhat with a subtle high-low-high-low-high-constant pattern covering 1979-1980-1981-1982-1983-1984. Calcium, which also did not show significant variation at the 95% confidence level, also appeared to subtly follow the general pattern of the other elements. The only exception here being an almost constant concentration level between 1981 and 1982 instead of a decrease as was exhibited by the other elements. Despite the lack of significant variation of some of the element concentrations, a recognizable pattern of temporal variation emerges nonetheless.

Profile analysis was again employed to test the assumption of similar fluctuation patterns. In this case, a log transformation was performed on the data, and they were transposed to produce groupings based upon year of sample production. The year groupings are displayed in Figure 15. As a whole, the groups by year varied significantly from one another ($p < .001$). This is interpreted as supporting the hypothesis that elemental concentration variation from year to year is not entirely uniform. Paired profile analysis tests, however, reveal that almost two thirds of all the possible combinations of years (23 of 36) do not show

Figure 15. Log plot of mean elemental concentrations by year of horn sample production as used in profile analysis.



significantly different elemental concentration variation from one another ($.051 < p < .994$). Except for the three paired combinations of 1979-1981, 1979-1982, and 1980-1982, all possible combinations from 1979-1985 were found to be parallel. This fact is interpreted as suggesting that a general pattern was followed by all elements between the years of 1979 and 1985. Figures 13 and 14 support this idea by displaying the fluctuation pattern that was described above. An additional profile test was performed on the sub-group of years 1979-1985, and as a whole these seven years were found to not vary significantly from one another ($p = .271$). Since this group of years was considered to be parallel, the next test of coincidence was performed. The results of this test indicate that the seven years do not all exhibit the same mean elemental concentration values, however ($p < .001$). These results support the previously mentioned interpretation. Elemental concentration fluctuations from year to year do vary significantly, but the pattern of fluctuation for a given time period is observed in all elements.

The only year that showed little correlation with other years was 1986. It exhibited significant differences from all other years except 1987. It was in 1986 that some of the elements were found in substantially higher concentrations relative to previous years while other concentrations of elements remained

fairly stable. Nonetheless, a fairly consistent pattern of annual elemental concentration fluctuation is present. In general, the study elements showed similar variation from year to year with respect to each other.

Temporal Variation at Lake Mead Only

In an effort to describe annual fluctuations of elemental concentrations more precisely, samples collected from Location 7, the Lake Mead area, were analyzed separately. This location provided the largest number of samples from a single area, while other pertinent data specific to the locale (eg. precipitation and plant mineral content) were also available.

Mean values and 95% C.I.s for elemental concentrations from the Lake Mead area based on year of sample production are found in Table 6. Analysis of variance revealed only two elements as exhibiting significant (95% confidence level) concentration variation with respect to year, phosphorus ($p=.0012$) and magnesium ($p=.0065$). The ANOVAs were performed both with and without the inclusion of data from 1982 ($n=2$, see Table 6), and the results of each set of tests were quite similar. Table 7 presents F-statistic and p-value results of ANOVA for the eight study elements with respect to temporal variation at Lake Mead, and includes data from 1982. Sodium ($p=.0600$) and potassium ($p=.0664$) were the only other elements to exhibit even

Table 6. Mean concentration values (ppm) and 95% C.I. for the eight study elements from the Lake Mead area with respect to year of horn sample growth.

ELEMENT	MEAN \pm 95% C.I.			
	1981	1982	1983	1984
Zn	112.3 \pm 17.5	110.0 \pm 24.7	113.9 \pm 17.5	96.4 \pm 11.7
P	935.0 \pm 204.3	630.6 \pm 289.1	886.8 \pm 204.3	686.4 \pm 136.3
Fe	56.2 \pm 31.4	65.3 \pm 44.4	72.6 \pm 31.4	57.5 \pm 20.9
Mg	253.3 \pm 34.2	247.7 \pm 48.4	270.9 \pm 34.2	213.9 \pm 22.9
Cu	2.2 \pm 1.2	2.6 \pm 1.6	3.6 \pm 1.2	3.2 \pm 0.8
Ca	630.9 \pm 123.6	892.4 \pm 174.7	733.3 \pm 123.6	715.4 \pm 82.4
Na	1140.4 \pm 207.0	909.6 \pm 292.7	1233.0 \pm 207.0	910.1 \pm 138.1
K	1743.1 \pm 492.3	1335.9 \pm 696.3	1751.6 \pm 492.3	1440.4 \pm 328.2
	n=4	n=2	n=4	n=9

ELEMENT	MEAN \pm 95% C.I.		
	1985	1986	1987
Zn	112.5 \pm 7.3	113.0 \pm 9.0	115.4 \pm 8.8
P	948.6 \pm 85.2	935.1 \pm 105.5	1057.4 \pm 102.3
Fe	56.8 \pm 13.1	45.5 \pm 16.3	62.9 \pm 15.7
Mg	263.7 \pm 14.3	271.3 \pm 17.7	266.2 \pm 17.1
Cu	2.4 \pm 0.5	2.7 \pm 0.6	3.2 \pm 0.6
Ca	693.9 \pm 50.9	684.1 \pm 63.9	665.9 \pm 61.9
Na	1146.9 \pm 86.4	1131.1 \pm 106.9	1085.6 \pm 103.5
K	1921.4 \pm 205.4	1799.4 \pm 254.3	2083.0 \pm 246.2
	n=23	n=15	n=16

Table 7. F-statistic and p-value results of ANOVA for the eight study elements with respect to year of horn sample development. All samples taken from the Lake Mead area only.

<u>ELEMENT</u>	<u>F-ratio</u>	<u>p-value</u>	<u>d.f. total</u>
Zn	1.255	.2905	72
P	4.202	.0012	72
Fe	0.592	.7358	72
Mg	3.313	.0065	72
Cu	1.315	.2629	72
Ca	1.274	.2816	72
Na	2.142	.0600	72
K	2.087	.0664	72

closely significant variation. The remaining four elements all produced p-values $> .25$. A partial explanation for the lack of significant variation may be found in the fact that mean values for 1981-1983 were all calculated from relatively small samples (see Table 6.). This has the effect of increasing the error term and thus decreasing the value of the test statistic (Zar, 1984).

Despite the fact that most of the elements did not exhibit significant temporal variation at Lake Mead, many of the elements still exhibited similar patterns of concentration fluctuation. The elements were again divided into high and low concentration groups for clarifying interpretation. The low concentration group which ranges from 2-271 ppm includes Zn, Fe, Mg, and Cu, and is presented in Figure 16. The high concentration group which ranges from 631-2083 ppm includes P, Na, Ca, and K, and is presented in Figure 17. All element concentrations decreased at least somewhat from 1983-1984. This is the only pattern demonstrated by all eight elements, however. The most notable pattern of concentration fluctuation occurs between 1981 and 1985. Five of the eight study elements (Zn, Mg, P, Na, and K) decrease in concentration from 1981 to 1982, increase in 1983, decrease in 1984 and increase in 1985. The two lower concentration elements (Zn and Mg) display only subtle changes, while the three

Figure 16. Mean concentration plots for zinc, iron, magnesium, and copper by year of horn sample production for location 7, the Lake Mead area only.

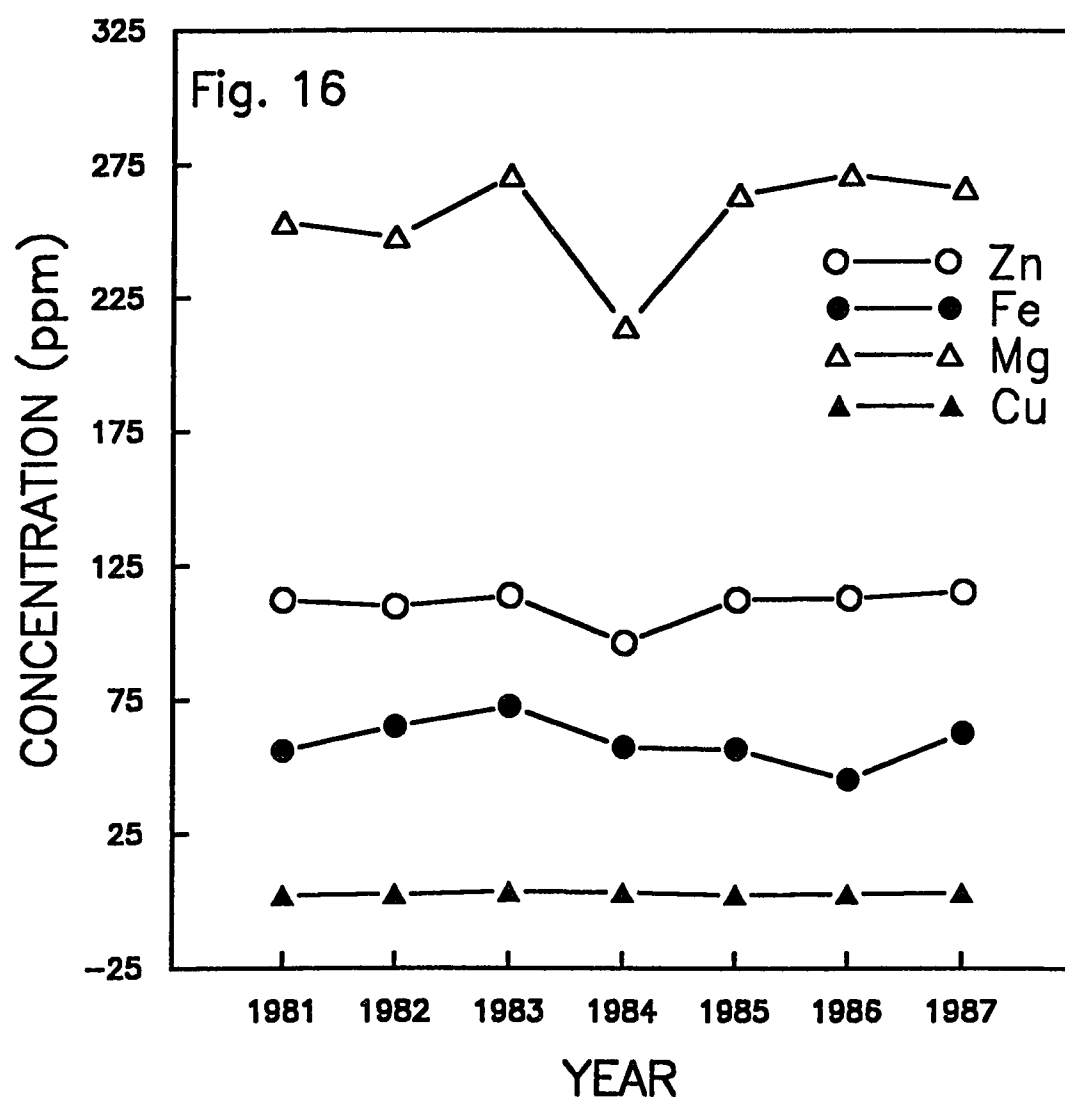
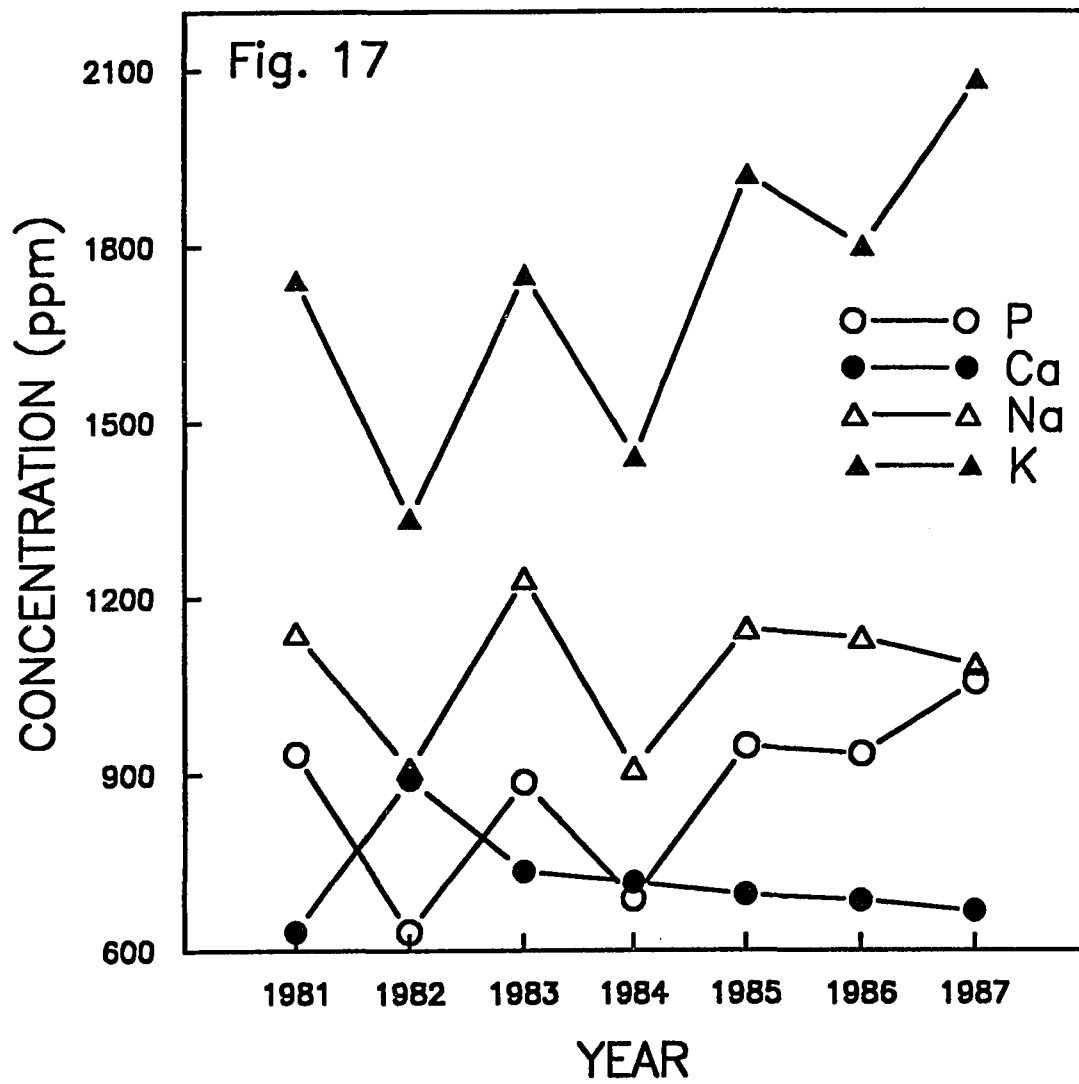


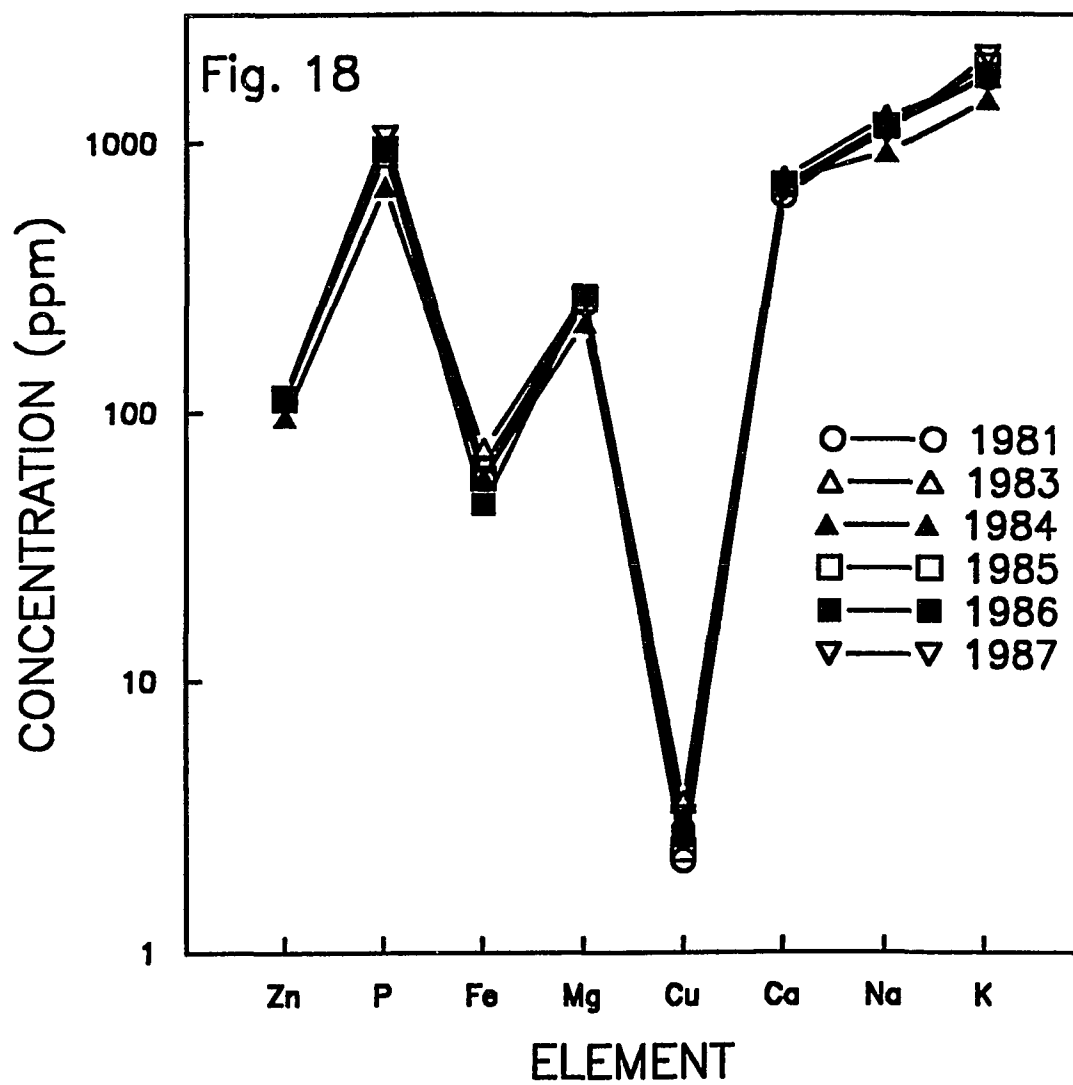
Figure 17. Mean concentration plots for phosphorus, calcium, sodium, and potassium by year of horn sample production for location 7, the Lake Mead area only.



higher concentration elements (P, Na, and K) exhibit fairly dramatic annual fluctuations. Copper and calcium concentrations appeared to vary quite independently from the concentrations of the other five study elements. The Lake Mead area data were transformed and transposed in order to perform profile analysis, and they are displayed graphically in Figure 18. Because of the small sample sizes of 1982, the data for this grouping were omitted prior to performing profile analysis. The six remaining years were shown to exhibit significantly different patterns of mean elemental concentration fluctuation when analyzed together ($p = .036$). Paired profile analysis tests revealed that 1984 differed significantly from all other years, but at the same time, the five remaining years were all significantly parallel with each other ($.104 < p < .595$), with the one exception of 1981 and 1986 ($p = .008$). Again, a general pattern of annual elemental concentration fluctuation appears to be present. In years when the concentration of any one element is relatively high or low, the concentrations of other elements will likely be relatively high or low respectively as well.

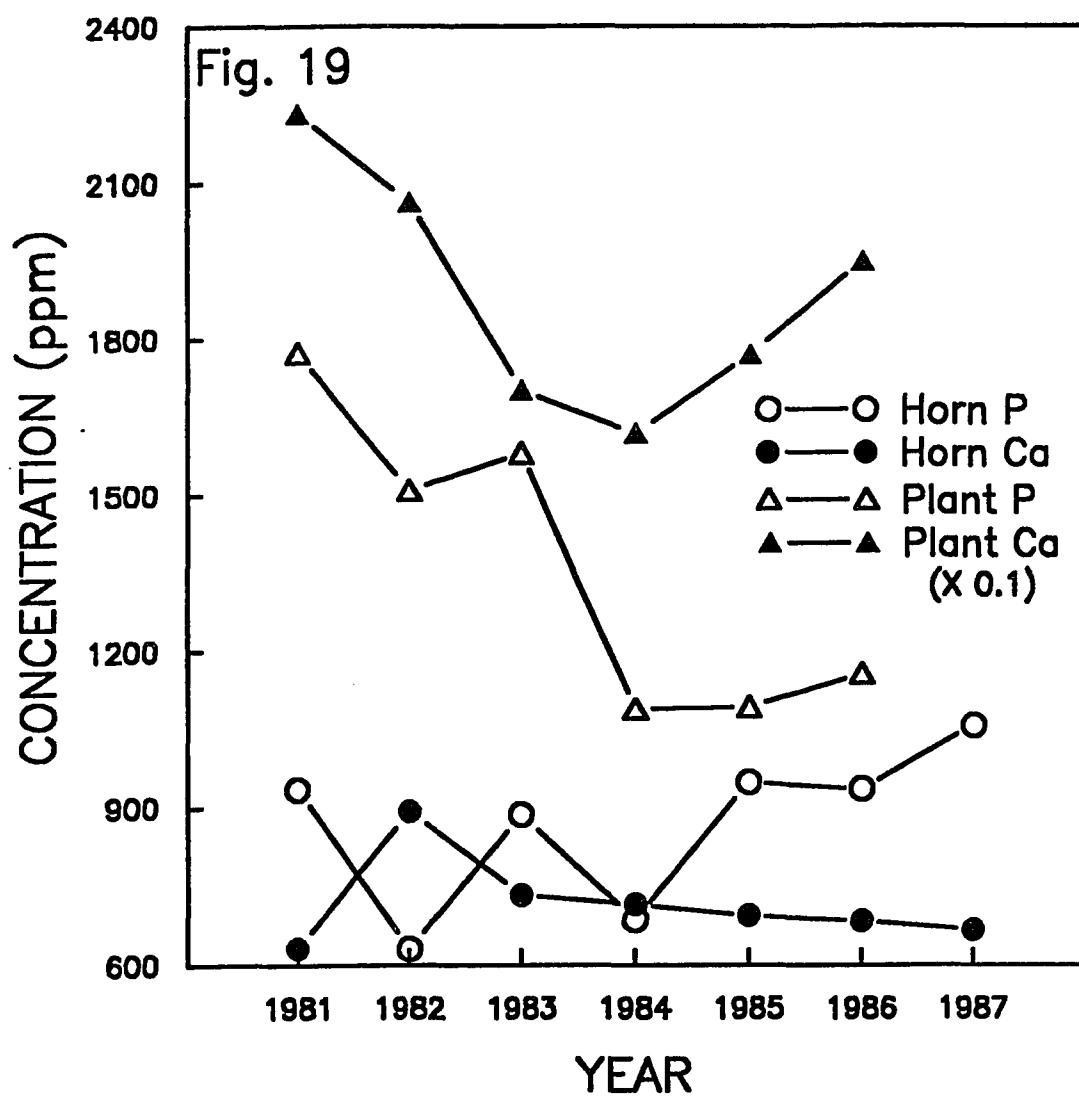
Although the patterns of fluctuation of element concentrations from samples taken from the Lake Mead area are not as widespread as is seen when all samples are analyzed as a whole, the pattern is still recognizable and worth investigating.

Figure 18. Log plot of mean elemental concentrations by year of horn sample production for location 7, the Lake Mead area only as used in profile analysis.



Data on phosphorus and calcium concentrations in plants from the Lake Mead area were available from ongoing studies conducted through the Cooperative National Park Resources Studies Unit at the University of Nevada, Las Vegas. Mean value indices were calculated for plant P and Ca and plotted simultaneously with mean P and Ca concentration values from horn samples (see Figure 19). Note, that in order to plot plant Ca simultaneously with the other elements, its mean concentration values had to be divided by a factor of ten. It is quite possible that the plant Ca values are highly inflated due to external contamination from airborne CaCO_3 (Lattman 1973, Pewe et al. 1981). Phosphorus appeared to provide a good representative fluctuation pattern for most of the elements analyzed from horn samples from the Lake Mead area (see Fig.17). It was assumed that horn and plant element concentrations would be relatively tightly correlated, as the main source of elemental acquisition would be diet (Hopps 1977, Peereboom 1985, Byrne and Paris 1987). Horn and plant P do not appear to be strongly correlated when their concentration variation patterns are viewed together (Figure 19). Horn P concentration fluctuations seem to only be paralleled by plant P fluctuations when they are decreasing, as between 1981-1982 and 1983-1984. This relationship is tenuous at best. Horn and plant Ca concentrations also do not appear to be related. Again,

Figure 19. Mean concentrations of horn phosphorus, horn calcium, plant phosphorus, and plant calcium by year for location 7, the Lake Mead area only.



environmental contamination may be affecting plant Ca values and rendering comparisons ineffective. Except for the general decrease in both plant P and plant Ca from 1981-1984, clear pattern repetition is not present. Despite the assumption of plant mineral and horn mineral relatedness, there appears to be no strong relationship. A possible explanation for this disparity is presented in the next section.

Assuming still that a plant - horn elemental relationship may exist, but was just not found, annual precipitation data for the Lake Mead area were studied also. Data were obtained from the Lower Colorado Regional Office of the U. S. Bureau of Reclamation at Boulder City, NV. It was felt that the ultimate control of annual fluctuations of element concentration at all levels of the system may lie in precipitation events prior to and during spring. Other studies have clarified the relationships between autumn rains and spring plant growth (Beatley, 1969), and also the relationship with bighorn sheep lamb survival (Douglas and Leslie, 1986). After several precipitation regimes were investigated, it was found that previous October - current January precipitation varied somewhat similarly with horn P concentrations. Horn P concentration was again chosen as a typically fluctuating element for the Lake Mead area, and its annual concentrations are plotted along with mean monthly precipitation for Oct.-

Jan. (Figure 20). Both horn P and precipitation exhibit similar patterns of annual increase and decrease from 1981-1986, but the coincidence fails from 1986-1987. A profile analysis test was conducted to determine the significance of the precipitation - horn P relationship, and no significant similarity was found ($p = .002$). Since the possible relationship was hypothesized to be causal, a regression analysis test was also conducted. The results of this test also support the conclusion of no significant relationship between precipitation and horn P ($r = -.468$, $p = .289$).

Correlation of Horn and Hair Elements

Although hair samples were collected from as many sheep as possible from the Lake Mead area, only eleven samples were compared with horn samples from the same animal for the purpose of correlation analysis. These eleven animals were all lambs of 1987, and therefore all produced hair and horn samples of the same year. Table 8 provides mean elemental concentrations and standard errors for both horn and hair samples. Zinc, P, and Na were all found to occur in significantly greater concentrations in horn samples when compared to hair samples using one-tailed t-tests (Zn, $t=2.41$, $p=.013$; P, $t=7.57$, $p<.0001$; Na, $t=4.67$, $p<.0001$). Iron, Cu, Ca, and K were all found to occur in significantly lower concentrations in horn samples when compared to hair

Figure 20. Mean concentration of horn phosphorus and mean monthly precipitation for previous October - current January by year for location 7, the Lake Mead area.

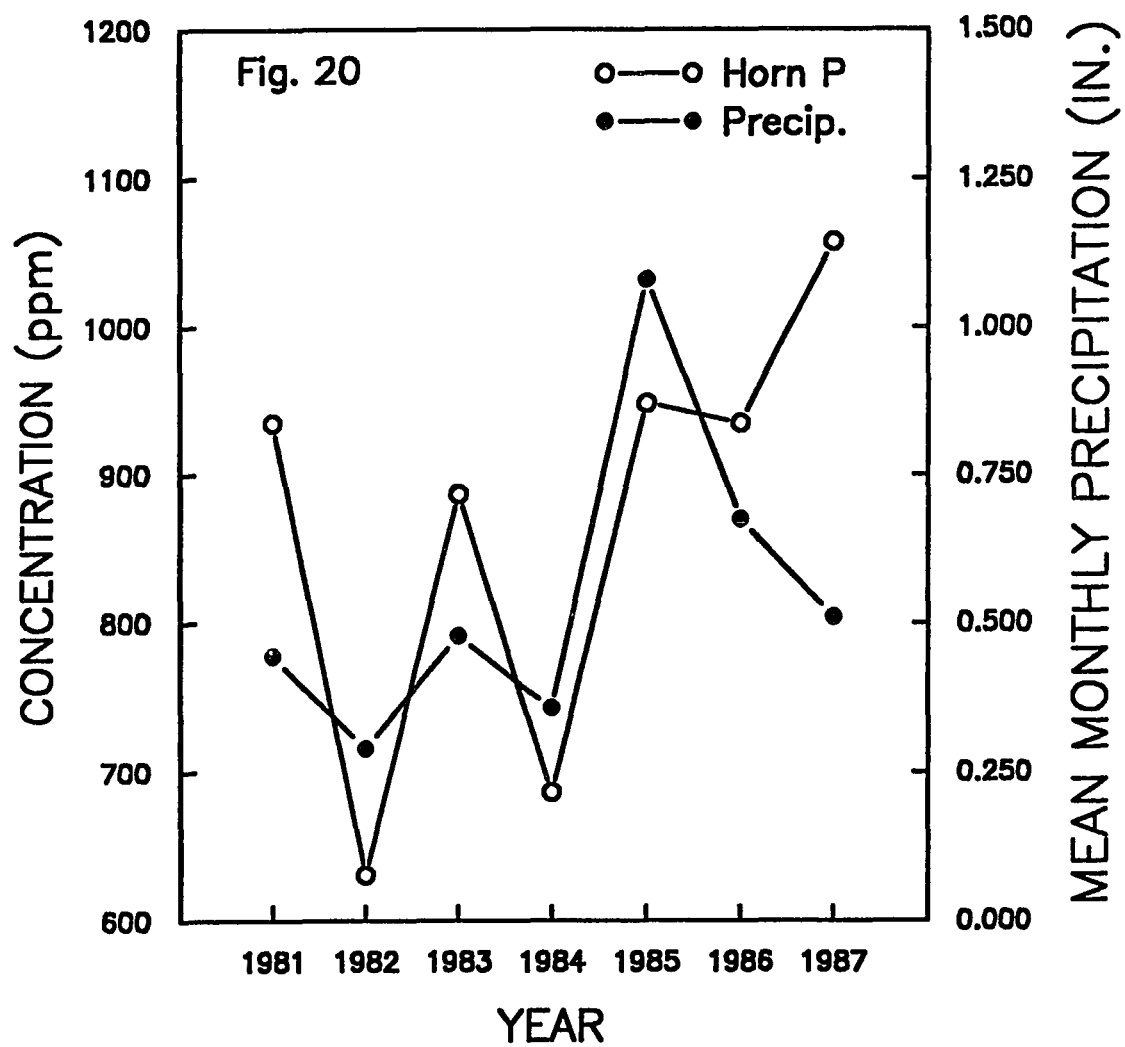


Table 8. Mean elemental concentrations (ppm) and standard error of horn and hair samples from eleven bighorn sheep from the Lake Mead area.

<u>ELEMENT</u>	<u>HORN</u>	<u>S.E.</u>	<u>HAIR</u>	<u>S.E.</u>
Zn	115.0	7.7	94.3	3.8
P	1026.2	66.9	337.1	61.7
Fe	71.2	20.2	254.2	101.9
Mg	258.5	9.9	427.8	114.0
Cu	3.0	0.5	11.7	3.8
Ca	678.1	51.1	2047.7	406.3
Na	1114.1	77.4	527.8	99.0
K	2067.5	93.0	4244.3	640.4

samples using one-tailed t-tests (Fe, $t=-1.76$, $p=.047$; Cu, $t=-2.30$, $p=.016$; Ca, $t=-3.34$, $p=.002$; K, $t=-3.36$, $p=.002$). Magnesium was the only element found not to occur in significantly different concentrations in horn and hair samples ($t=-1.48$, $p=.077$).

Correlation analysis revealed that none of the elements correlated significantly with themselves (eg. horn Zn and hair Zn) when horn and hair samples were analyzed, (Table 9). The entire correlation matrix (16X16) did, however, reveal a few significant relationships between different elements from the same sample material (eg. horn Zn and horn P, or hair Fe and hair Mg), but the significance of these relationships was not pursued. The main finding of this portion of the study lies in the lack of correlation of horn and hair element concentrations.

Table 9. Correlation coefficients and p-values from correlation analysis of horn samples with hair samples. Results only represent analysis of like elements from samples from eleven animals. Note, all correlations are non-significant.

ELEMENT	Coefficient (r)	p-value
Zn	.2049	.5456
P	-.0886	.7955
Fe	.0462	.8926
Mg	.1058	.7569
Cu	-.3217	.3347
Ca	.0524	.8784
Na	.4265	.1908
K	-.2521	.4546

DISCUSSION

Geographic Variation

Results of the investigations of geographic variation of the eight study elements indicate that, individually, every element measured from sampled horn material occurs at significantly different concentrations between locations. The main reason for this generally, is the significantly different concentration in which a given element is found at one or two locations in horn material as compared with the remaining seven or eight locations taken as a group. Recall that individual multiple range tests indicated that six of the eight study elements separated into groups based on location, where one of the groups only consisted of one or two locations. This explanation holds for P, Mg, K, Fe, Ca, and somewhat for Na, but not for Zn or Cu. This relationship can be seen in Figure 12. Except for points at Zn and Cu, the plot for location 7 lies above all the other plots and is coincident with the plot for location 1 at Ca. This relationship may not have a clear explanation, however. There seems to be no definite reason why so many of the elements occur at higher concentrations at one

particular location when compared to other locations.

Indeed, some small groups of locations were shown to exhibit similar element concentration profiles in sampled horn material, but as a whole (or in larger groups) the locations exhibited relatively unique element profiles. This should be expected. If horn samples reflect local conditions in any way, one would not expect differences to be based solely on spatial separations. Physical differences of habitats of different locations is a more plausible delineator. This view is supported by Bartolotti et al. (1989). They found no association between element profiles of spruce grouse feathers and geographic locale, but they did find that grouse from forests of similar vegetation composition had feathers with similar mineral profiles. They employed discriminant function analysis (DFA) to determine whether element profiles were stand specific. The stands were first defined by an ordination technique, and then DFA was used to examine the relationships of feather mineral profiles from habitats that had been defined as similar. By grouping similar feather mineral profiles, they were able define habitat parameters that were most closely correlated with differences in feather elements.

The approach taken by Bartolotti et al. (1989) may be applicable to this study. Habitat information for the nine locations defined in this study could be used

to define groupings of samples different from the purely spatial groupings used here. In addition, habitat ordination of the nine locations may help explain why some locations exhibited similar element concentration patterns in sampled horn material while some locations appeared to be fairly dissimilar. Variables used in any habitat ordination would probably need to include both vegetation factors and geologic factors. Bartolotti et al. (1989) stress in their paper on spruce grouse feather analysis that "multivariate statistics, as used [in their study], are almost mandatory in studies of mineral profiles". Future studies of horn element profiles may well benefit from the use of such analysis procedures.

Temporal Variation

This study provided evidence in support of the hypothesis that annual variation of element concentration is fairly consistent among the different elements. When all samples were examined for annual fluctuation similarities, a group of seven years (out of nine) was found to exhibit significantly similar patterns of element variation. When samples from location 7 were examined separately, a group of five years (out of six) was found to exhibit significantly similar patterns of element variation as well. Although the relationship does not hold for all possible cases,

it does appear prominently enough to conclude that variation of horn element concentration from year to year is similarly proportional among the different elements. As stated earlier, the relative annual concentration fluctuation of any one element is generally imitated by other elements.

The relationship of temporal variation among the different elements suggests the presence of some type of controlling factor. This study investigated a hypothesized relationship between plant elements and horn elements. Despite the assumption of plant mineral and horn mineral relatedness, there appears to be no strong relationship. A possible explanation for this disparity may be found in the fact that not all plant samples, which were collected between 1981 and 1986 were analyzed for mineral concentration information. This produced mineral concentration data that did not cover the same time periods each year. Plant P concentrations, for example, are known to be higher during the spring when it is translocated to growing tissues from the roots (Chapin 1980). Samples from 1982 were analyzed for every month from January - November, while samples for 1981, 1983, and 1985 were analyzed only for October - December (1985 also included August and September), and samples from 1984 and 1986 only included analysis for the spring and summer (January - June, and January - September respectively). Perhaps if

the plant data had been uniform, a stronger relationship with horn concentration data would have been apparent. These problems could be corrected and the relationship investigated again. An additional improvement in investigating the plant - horn element relationship would be to employ plant mineral analysis methods which are more like the ones used for the horn analysis. Not only would this standardize the analytical procedures, but it would allow the plant samples to be analyzed for all the same elements as horn samples. The relationship of horn elements and plant elements was only intended as a small component of this study, but in the light of the strong temporal variation patterns that have emerged, a more detailed investigation of annual variation of plant element content may be warranted in the future.

A component of annual precipitation was compared with annual horn element content, and no relationship was discovered. Although the results of this portion of the study were negative, it still seems plausible that some environmental factor, such as precipitation, must be related to the consistent temporal concentration variation seen in horn samples. Only one variation of many possible precipitation regimes was investigated in this study. This regime was derived in part based on evidence provided by other investigators that previous fall precipitation strongly influences growth of spring

annuals (Beatley 1969) and bighorn sheep lamb survival (Douglas and Leslie 1986). Investigating other precipitation regimes may help in discovering a relationship that ties horn element content to an environmental factor. Perhaps precipitation works in conjunction with other factors such as temperature or humidity, and a better indicator of horn element variation may be found in variables such as soil moisture, or ambient humidity. These relationships could all be investigated, but were beyond the scope of this study. The main finding here lies in the discovery of the similarity of variation of annual horn element concentration between elements.

Correlation of Horn and Hair Elements

This study failed to reveal a significant correlation between horn and hair element concentrations. Although metabolic mechanisms may well be responsible for disproportionate depositions of minerals, this explanation is difficult to investigate. One needs first to rule out the possibility that one or both of the sample types may be contaminated. Hair for instance appears to be relatively subject to exogenous contamination (DeAntonio et al. 1982). Gibson and Gibson (1984) feel that "after careful laboratory washing, the effects of adventitious contamination on hair trace element content are small, and can be

effectively ignored". If this is true, a slightly more vigorous washing procedure may help clarify any possible horn - hair element correlations, if they exist.

Hilderbrand and White (1974) agree that hair element measurements can be greatly affected from external sources, and they also believe that commonly used washing procedures are not effective in correcting for the contamination. Despite the discrepancy of these two opinions, the same correlation analysis could still be performed with additional washing of hair samples. If still no correlation was found, one would conclude that regardless of the cause, hair and horn samples simply cannot be compared. If a significant correlation was found, one could conclude that contamination was the main problem during the present analysis.

CONCLUSION

Although trace element studies have been conducted involving numerous types of sample materials and various species, work on bighorn sheep horn material is fairly scarce. This study showed that element content of horn exhibited significant variation both spatially and temporally. The relationships with environmental factors explored here were insignificant, but the opportunity for future related studies in this area may be promising. Problems associated with some of the aspects of this study could be accounted for, and future studies involving variations on similar themes could provide useful information.

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