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COMPLETION REPORT

TITLE: Elemental Composition of Statoliths of Sea Lamprey (*Petromyzon marinus*)

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Abstract: Elemental Composition of Statoliths of Sea Lamprey (Petromyzon marinus)

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Elemental composition of the statoliths of sea lamprey (Petromyzon marinus) was investigated as a means of stock identification required for lamprey control in the Great Lakes. The goal of the investigation was to explore the feasibility of trace element analysis to discriminate the relative proportions of adult lampreys which have spent their larval life in different river systems. The statoliths, although small (maximum diameter - 350µm; 30µgm per individual), are the only calcified (apatite) structures present throughout larval life and show little additional growth in the adult phase. Six analytical techniques were employed to test for useable trace element chemoprints in the ammocoete lamprey statoliths from five localities (Canada and New York). Comparative materials also examined included various stream and lake waters, NIST certified bone samples, and trout otoliths. "Non-destructive" analyses were carried out on individual statoliths (energy dispersive and wavelength dispersive spectrometry of electron microprobe excited x-ray fluorescence, EDS and WDS) or pooled samples from several individuals (x-ray excited fluorescence spectrometry; XRF). "Destructive" preparations involving acidic dissolution of pooled samples (2-40 statoliths; 1-20 individuals) were subjected to neutron activation analysis (NAA), and inductively coupled plasma atomic emission or mass spectrometry (ICP-AES and ICP-MS).

The XRF method was capable of detecting Ca and Zn and possibly Fe, but was insufficiently sensitive to other trace elements, particularly in the presence of strong background peaks produced by the sample cups. Even with elimination of this artifact, the instrumentation would not be able to detect the expected concentrations elements in samples of only two statoliths. EDS and WDS analysis demonstrated the presence of Ca, P, Mg, Cl, K, and Na. Detectability of the latter element was questionable with EDS, but positively confirmed with WDS. Differences between localities could not be demonstrated with these methods. Magnesium was found to be distributed in a spatially heterogeneous pattern, possibly associated with presumptive annual zones. NAA revealed the presence of Ca, Cl, Mg, Na, K, and Mn, but the small sample size represented by two statoliths is a significant problem for detecting and quantifying elemental composition by NAA. ICP-AES analyses clearly demonstrated the presence of Ca, P, Mg, Mn, Fe, and Na. Results were reproducible and observed values agreed with expected concentrations for samples of known composition. There were indications of consistent differences between some localities. Modification of the instrumentation to allow for smaller sample volumes would substantially improve sensitivity. The ICP-MS is generally one to three orders of magnitude more sensitive than the ICP-AES. Contamination artifacts are therefore

proportionally more significant and may obscure results for some of the less abundant elements. Quantitative comparisons of ICP-AES and ICP-MS results for the more common constituents (Na, Mg, P, Cr, Fe, Mn, Zn, and Pb) gave agreement within an order of magnitude or less. Although there was only a limited number of samples available for analysis, there was generally good similarity between different samples taken from the same site. Distinctions between sites were either not obvious or possibly influenced of contamination. Site-specific differences in Mn, Fe, Zn, Na, and Cu looked most promising. Future studies should employ a direct injection nebulizer, laser ablation or electrothermal vaporization to reduce sample dilution.

It is concluded that positive indications in the results support the need for additional analyses, particularly with newer ICP techniques and greater emphasis on reducing possible contamination.

INTRODUCTION

The identification of fish populations or elucidation of finer levels of population structure is often a necessary prerequisite to enlightened fishery management. Many methods or tools are available to fisheries scientists for this purpose, including: fish marking; "classic" meristic and morphometric analysis; "natural tags" such as diseases or parasites; life history and behavior studies; cytotaxonomic characterization; electrophoretic mobility of proteins; serology; and analysis of chemical composition, particularly of "trace" elements in calcified structures and other tissues (Everhart et al., 1975; Ihssen et al., 1981). Important activities of the Great Lakes Fishery commission include monitoring and control of sea lamprey populations in the lakes and their tributaries (Fetterloff, 1980). Although ammocoete surveys may indicate the abundance of the larval stage in tributaries, more intensive collecting efforts, such as trapping of transformers, are needed to estimate the relative contribution of particular rivers and streams to the adult lake "population". Major unknowns in lamprey biology include the dynamics of dispersal, stream fidelity, and the potential importance of "alternate sources of animals that re-populate streams after chemical control" (Walters et al., 1980). How can one identify the natal stream of an adult lamprey collected in a lake or as an upstream migrant? Attempts to apply both traditional and new methods of population identification to lampreys are either frustrated by this animal's unique biological characteristics, or have met only very limited success. For example, electrophoretic analysis (Kreuger and Spangler, 1981; Wright et al., 1985) has demonstrated consistent differences between some lake populations and even streams, however for the most part these differences are statistical distinctions based on frequencies of certain alleles. This sort of analysis cannot be used to identify the home stream of individuals with certainty, and presents more problems when adult lake collections are made, since these lampreys are a "mixed" group from many tributaries. Electrophoretic distinctions between streams are generally blurred. The only really useful electrophoretic characters are unique alleles or fixed (or nearly fixed) alleles, which alone or in combination may serve to identify the natal stream of a lamprey. Such characters, although present, appear to be fairly uncommon and not generally available to identify the majority of possible lamprey sources.

The analysis of the chemical composition of fish tissues is a promising and potentially powerful technique to be used in identifying populations. The basic premise is that many materials are incorporated in small amounts in the tissues as traces and that the relative and absolute deposition rates and resultant concentrations of these materials is a function of local environmental availability. Furthermore, it is assumed that once deposited, the mix of trace materials is not substantially altered, and chemical composition may be analyzed to yield a "chemoprint" or locality specific compositional profile. In practice the materials to be analyzed are at the elemental level (although theoretically various compounds, including many types of pollutants, could also be examined) and may be referred to as trace elements, since the ones of greatest interest are likely to be in low concentrations. These elements may or may not be biologically active, in fact the ones of lesser activity are more significant to the analysis since they are less likely to be metabolically transferred in or out of tissues or turned over. Several studies have carried out trace element analysis for the purposes of stock or population identification; some have looked at all detectable elements and statistically compared multiple peaks in x-ray spectra (Calaprice, 1971: Mulligan et al., 1980) while others have concentrated on one or very few elements of interest such as zinc, iron, strontium or certain rare earth elements (Miller, 1963; Papadopoulou et al., 1978, 1980; Gauldie and Nathan, 1977; Bagenal et al., 1976; and D. Martin, pers. commun.). In all of these examples the investigators were successful in discovering compositional differences in otoliths, scales, bones, or whole organisms which were correlated with locality of collection. It should be noted that the relative tissue concentrations of many of these elements is a function of availability in the environment plus temperature mediated physical and biological processes, i.e. the rate of incorporation or substitution in tissues is a temperature dependent process. A complete understanding of these phenomena may allow for back-calculations of temperature from elemental composition or deductions of spatial distribution and origin (e.g. Degans et al., 1969; Gauldie and Nathan, 1977; Schneider and Smith, 1982; Radtke and Targett, 1984). However, temperature effects can also confuse or obscure analysis for stock identification by "compensating" for differences in availability and seasonal fluctuations. A good example is the seasonal change in magnesium content in the shells of bivalve molluscs (Rosenberg, 1980).

To date, most chemical analyses of fish calcified tissues have been done on marine fishes (see also Dannevig, 1956); organisms which are exposed to a relatively rich and diverse chemical bath - seawater. For example, otolith concentrations of elements such as iron, magnesium, manganese, strontium, sodium, and zinc range from a few to several thousand ug/g (ppm). Exposure and incorporation levels in freshwater fish is likely to be considerably lower. Finally, it must be pointed out that for the application under consideration here, sea lamprey, the goal is to identify the natal stream where larval (ammocoete) growth took place. Adult lamprey are considerably larger than the larvae, or even a transformer. Thus the vast majority of tissue present in an adult has been formed while the individual was in the parasitic lake phase. Trace element availability during this stage is a function of lake water chemistry plus the food source, a variety of fishes, each with its own complex life history and variable exposure to trace elements. There is little point in analyzing adult tissues to determine their stream of origin, unless that tissue or material can be demonstrated to have been formed in the larval phase and to have remained unchanged since that time. There is only one such material in a lamprey, the ear stones, specifically the statoliths (Carlstrom, 1963; Volk and Brothers, unpublished ms). There are no other calcified tissues in a lamprey, and our best evidence indicates that the statoliths represent permanent records of early growth and are not resorbed or chemically changed during the life of an individual. This may or may not be true of the otoconia, the smaller and more numerous calcified bodies also in the ear.

The statoliths of sea lamprey offer several advantages and disadvantages as subjects for elemental analysis for the purposes of population identification. First, lamprey larvae are long lived (at least 5 years), burrowing, filter-feeding organisms in the sediment of streams (Moore and Mallatt, 1980). They are in intimate contact, via respiratory and feeding activity with large volumes of water and associated dissolved and suspended materials. Therefore they have ample opportunity and time to contact moderate quantities of even scarce elements (Leppard, 1983). The statoliths appear very early in the life of the lamprey; several weeks after hatching (pers. obs.) and grow throughout the larval period by the addition and incorporation of successive layers of otoconial material (Volk and Brothers, unpublished ms). This additive or growth process takes place on the anterio-ventral

aspect of the statolith (the "flat" side), and therefor there is a layered internal structure which corresponds to a temporal record. The statoliths, although small (maximum diameter ca.350 um; mass - 10 ug; Fig. 1 and Fig. 2), are composed of calcium phosphate as apatite. Mineral and biological apatites are typically rich in trace elements (Dr. Robert Kay, Dept. Geology, Cornell Univ.) and so the statoliths are likely to contain measurable levels of several trace elements. Furthermore the statoliths show little or no additional growth after metamorphosis. This means that chemical analysis of whole adult statoliths is probably equivalent to looking at larval (stream) deposition with an insignificant contribution from the adult (lake) phase. As a further check on this assumption, electron microprobe analysis techniques can allow separate determinations for different portions of an individual statolith, so material deposited during the larval stage can be directly compared with the last deposited apatite from adult lampreys (Fig. 3).

Several analytical methods may be used to characterize the trace element composition of sea lamprey statoliths. Each has different characteristics with respect to sensitivity, range of elements analyzable or surveyed at a time, ease of sample preparation and analysis, need for sample dissolution or destruction, sample size requirements, and ability to spatially resolve elemental distribution. The research described here utilized five analytical techniques to test the feasibility of trace element fingerprinting or chemoprinting in sea lamprey statoliths: X-Ray Fluorescence Spectrometry with the electron microprobe (Energy Dispersive Spectrometry, EDS; and Wavelength Dispersive Spectrometry, WDS); X-Ray Excited (tube) Fluorescence Spectrometry (XRF); Inductively Coupled Plasma Atomic Emission Spectrometry (ICP); and Neutron Activation Analysis (NAA).

Two possible approaches exist for stock identification using trace element analysis. One method relies on a broad survey of all detectable elements and the production of a spectrum or similar graphical display of the presence and relative abundance of these elements. The elemental composition of different individuals and/or populations can then be compared by a statistical analysis referred to as pattern recognition. This analysis can be fairly complicated and give ambiguous results if the compositional differences between samples is not clear-cut and results from relatively small differences in the abundance of several elements. Demonstrating the statistical significance of the differences would be expected to be difficult due to very

low levels of trace elements (close to the detection limits of the analytical procedures). A "best case" scenario would be if there were significant patterns of presence and absence (or high and low concentrations) of several elements, i.e. if certain populations deposited unique or signature elements. Even if this does not occur, a broad survey or chemoprint analysis will serve to identify the presence of any elements which are potentially useful markers for a more detailed quantitative analysis. This leads to the second approach: quantitative determination for one or a few elements of interest which show population-specific differences in incorporation rate. Statistical treatment of this approach would be simpler, however the analysis will require careful validation with standards. A relative quantification (e.g. with respect to a common element such as calcium) could possibly be used instead of absolute determinations of concentration.

MATERIALS AND METHODS

Statolith material was obtained from sea lamprey (Petromyzon marinus) ammocoetes collected at five localities in Canada and New York. These 1985 and 1986 collections were made during lampricide treatments with TFM or granular Bayer '73. Whole animals from all the collections (except the Cayuga Inlet) were frozen for approximately one year before being thawed and transferred to 95% ethanol. The Cayuga Inlet fish were never frozen but immediately fixed in ethanol. Collection data follow:

Locality	Date	Number Analyzed	Mean T.L.(cm)
St. Marys River (STM)	14 AUG 1985	57	11.3
Goulais River (GR)	22 AUG 1985	13	11.0
Little Salmon River (LS)	SEPT? 1985	21	11.4
Lindsey Creek (LC)	26 OCT 1985	13	12.3
Cayuga Inlet (CAY)	SEPT 1986	50	14.5

Statoliths were removed from the larvae by first making a complete transverse cut 1 - 2 mm behind the eyes. This bisects the otic capsules and leaves the statoliths in the membraneous tissues on the anterior side of the incision. The inner ear tissues were removed with fine forceps and placed in a drop of distilled water on a glass slide. The statoliths were then teased from the soft tissues with the aid of fine needles and a dissecting microscope. After air drying the statoliths could be picked up on the end of the needles by static charge. A rinse and dry cycle was then repeated and the statoliths were transferred to labeled gelatin capsules for storage.

Statolith mass was not directly measured as part of this study, but were approximated from a previously derived relationship of ammocoete length versus statolith mass for Cayuga Inlet samples (Volk and Brothers, unpublished); see Fig. 1.

SEM and Microprobe (EDS and WDS)

Statoliths were placed in silicon rubber molds and embedded in Spurr's mounting medium. After curing at 70 C overnight, the statoliths were ground and polished on their anterior face (the "flat" side") so as to produce a large smooth surface for analysis. Grinding was accomplished with dry Emery paper (grit 0000) and then a graded series of diamond pastes (3,1,1/4 um) on Mastertex cloth (all Buehler Ltd. products). Motorized polishing wheels were employed for all steps. The samples were then sonically cleaned in distilled water and then Freon. Polished specimens were vacuum coated with carbon and in a few cases gold/palladium sputter coating was tried as an alternative. Some samples were acid-etched before coating to improve Secondary Ion Imaging (SEI) in the SEM. The etchant was 0.01N HCl, and etching times ranged from 10 to 30 seconds.

EDS analysis was performed with an AMR-1000A microscope equipped with a Tracor-Northern x-ray detection and analysis system. SEI and BEI observations were made with a JEOL VSM-T200 microscope. WDS data were taken on a JEOL-733 Superprobe.

Samples of a reference standard calcined animal bone (Code No. A-3/1/1974) from the International Atomic Energy Agency were used as a standard for the Microprobe and other methods (see Table 1 for composition). Where

fixed, polished specimens were needed, a small amount of the powdered material was embedded in Spurr's medium and then processed as the statoliths were.

X-Ray Excited Fluorescence Spectrometry (XRF-EDS)

Two types of preparation were used for XRF analysis. Mounted and polished specimens as described above were placed on a Mylar film in the spectra-cup and centered in the beam path. This facilitated handling but necessitated running blanks of the Spurr's material. An alternate procedure was found to give comparable results without the added background contributed by the embedding medium. Statoliths in groups of 2 to 20 were placed directly on the Mylar material for irradiation. The instrument used for these analyses was an Ortec Tube Excited Fluorescence Analyzer (Model 6110, by EG&G Inc.). After running a number of samples and controls on this instrument it was discovered that the very small size of the statoliths was insufficient to block or absorb most of the beam's energy. As a result significant fluorescence was produced by the specimen holder; the plastic Spectra-cups. Strong energy peaks were produced for Ar, Ca, Fe, Cu, Zn, and Pb and this "background" almost masked the weaker signals from the statoliths. Alternate mounting procedures could not be attempted because the instrument was not operational during the latter part of the study. Most analyses were made with a beam energy of 40-50KV and an Mo anode and filter. Collimation of the beam gave only very slightly stronger peaks.

Neutron Activation Analysis (NAA)

Neutron Activation Analysis utilized the reactor and gamma-ray detection equipment of the Ward Laboratory of Nuclear Engineering (Cornell Univ.). Specimens were irradiated with thermal neutrons at a flux of approximately 400KW. Exposure times varied from 1 min to 1 hr. For different runs, counting began at 2 min to 1 wk after exposure. Counting times were at least 250 sec and most were 10 min to 24 hr. Most of the samples were counted several times over the course of a few days. Samples which contained high levels of elements such as Na or Ca produced interference with the gamma ray energies of trace elements and therefore could only be counted after the interfering short half-life isotopes decayed to acceptable levels. Background counts were made with an empty detector chamber and blanks were run with irradiated but empty specimen vials. Contaminants or composition of the

vials was a major concern due to the low levels of trace elements expected in the statoliths. Gelatin capsules and three types of polyethylene vials were tested and all showed substantial levels of detectable elements. In order to yield more useful results, later experiments were performed with irradiated statoliths and other samples being transferred to non-irradiated ("clean") vials before counting. For some runs the exposed materials were too radioactive to handle safely and they had to undergo decay for several hours to days before transfer. The criterion for designating the presence of a peak was a count per second (cps) value sufficiently above background to give a % error less than 20%. In most cases the % error was between 5 and 10% for the counting times used. Identification of elements from peak energies and calculation of relative concentrations was done with methods and tables in Skoog (1985), Bunker et al. (1986) and Weast, 1979.

Inductively Coupled Plasma Atomic Emission Spectrometry (ICP)

Statoliths were prepared for analysis by placing them in an acid-washed quartz test tube and then dissolving them in 0.4 ml of nitric acid. The fluid was then oven-dried before reconstituting a 1 ml solution in 5% HCl. This milliliter of solution was then analyzed with a Jarreal-Ash 975 Atom Comp. ICP. The same procedure was used for several trout otoliths and a bone standard that were also examined during the course of the study. Water samples of 1 ml were run directly without any modification. The otolith samples contained sufficiently high levels of calcium so that interference were significant. A known sample of 5000 ppm was run to calculate correction factors for these samples.

The approximate detection limits for this system are as follows (ppm):

Element	K	P	Ca	Mg	Mn	Fe	Cu
Det. Limit	1.45	.011	.045	.003	.0006	.002	.006

Element	B	Zn	Al	Na	Co	Cd	Cr
Det. Limit	.001	.0006	.002	.006	.002	.0016	.002

Element	Ni	Pb
Det. Limit	.016	.014

In general, concentrations 10x the detection limit are preferred to insure significance. All runs were background corrected.

Additional Samples

In addition to the statoliths and bone standard, two other types of materials were analyzed by some of the techniques. Two water samples were taken from the St. Marys River on 12 Dec 1986. One (labeled A) was collected downstream from major areas of industrial effluent; the other (B) was from upstream of these areas (see Fig. 2). The samples were analyzed with the ICP.

Otoliths from two trout were also analyzed with NAA and ICP to compare results on aragonitic material (otoliths) with the apatite of the statoliths. Two sagittae from a 315 mm S.L. rainbow trout (Salmo gairdneri) collected in the Madison river near Ennis Montana, were analyzed separately as replicates. The third sagitta was from a 620 mm S.L. bull trout (Salvelinus confluentus) collected in Flathead Lake, Montana.

RESULTS AND DISCUSSION

SEM

Polished statoliths showed no surface features in the SEI mode with the exception of cracking; an artifact present in most specimens. The outer margin of the sections indicated the spherical forms of the otoconia composing the statolith. Etching revealed the internal structure of the statoconia to be made up of fused otoconia (Fig. 3). More detailed views of the larger otoconial bodies located near the statolith apex (Fig.4) demonstrated that they grow in a lamellar fashion similar to what is known for teleost otoliths. There are central elements analogous to the primordium and core of otoliths (which are also visible as optically dense regions when viewed with transmitted light microscopy) surrounded by very fine layers which are differentially etched and therefore in three dimensional relief. The remarkable feature of these layers are their dimensions. The finest ones measured were less than 0.04 um thick. The layers appear to be structurally analogous to daily growth increments in teleost otoliths. Assuming that they are daily in nature allows for some very rough calculations of the period of formation for otoconia. Otoconia vary in diameter from about 0.3 to 25 um. If an "daily increment" thickness is taken to range between 0.1 and 0.03 um,

then the average period of formation for individual statoconia would be about 3 to 4 months.

SEM examination of polished statoconia in the BEI mode revealed some of the otoconial substructure, as well as areas of lower average atomic number. These regions of lower back-scattered electron yield appear darker in the photographs. Fig. 5 illustrates these features in their typical peripheral location. X-ray analysis (see below) indicated that these are regions of relatively higher Mg (atomic number 12) versus Ca (atomic number 20) content. This phenomenon was further explored since such regional variation was unexpected and could be caused by at least a few possible mechanisms. One possibility was that it was an artifact of the freezing, alcohol preservation or some other procedure in handling. Perhaps there was some leaching or substitution of Mg for Ca during the long period of storage. Comparisons of lampreys from different localities and with different storage times (the Cayuga Inlet fish were captured a year later) and handling procedures (some were not exposed to alcohol) seemed to show that it was a universal occurrence, but variable between individual statoliths and sections. A second hypothesis was that these regions appeared as the result of sectioning planes at oblique angles to some gross internal layering. An obvious candidate for this layering are the presumed annual zones noted in the statoliths (Volk and Brothers, unpublished ms). Furthermore it is known that some some invertebrate aragonites and calcites show a positive relationship between temperature and/or growth and Mg content. Seasonal cycles are sometimes clearly expressed (Rosenberg, 1980). More work is needed to elucidate the three dimensional features of the Mg rich zones in the statoliths, but one sagittally sectioned BEI view (Fig. 6) showed a zonation pattern consistent with the annulus hypothesis. The variation seen when the statoliths are sectioned or ground approximately parallel to the flat surface could be due to slight inconsistencies in mounting position or cutting angle.

SEI and BEI examination of the calcined bone standard revealed a very non-homogeneous mixture of bone fragments combined with some small metallic-like particles (Fig. 7). The documentation with the sample stated "The chromium level is about 100 times greater than normally observed in this kind of material. The metal was probably introduced during the preparation." WDS analysis demonstrated that the metallic particles were chromium, iron, copper, and nickel. The most probable explanation is that the milling machine or some

other preparatory tool contributed flakes and chips to the bone sample. This was an unfortunate happenstance but provided graphic evidence for the power of the SEM and microprobe to spatially locate and identify elements within very small samples.

EDS

The detection limits for an EDS analysis system comparable to the one used in this study are shown in Fig. 8. Operating conditions which will modify sensitivity such as specimen thickness and roughness, accelerating voltage, spot size and counting time were controlled to give a range of sensitivity of 0.1 to 3% for elements of atomic weight = or > 11 (Na). In ppm this is equivalent to 1000 to 30000 ppm. Elements with atomic weights between 20 and 30 had the lowest detection limits

Analysis of the Spurr's embedding medium showed a single peak for chlorine (Fig. 8a). When an area which included both bone particles and surrounding epoxy was scanned the resultant spectrum showed the presence of P, Cl, and Ca (Fig. 8b). Spot analysis of a bone particle showed only the Ca and P apatite constituents (Fig. 8c). The contaminating metallic particles referred to above were not examined with the EDS system.

Fig. 9 how several EDS spectra for different lamprey localities and replicates for St. Marys specimens. The only significant peaks were for P and Ca. There is a very slight suggestion that the St. Marys statoliths had higher levels of Cl, but this element is of lesser interest because of its commonness and higher probability for being introduced as a contaminating artifact. Very long counting times of up to 20 min did not result in the emergence of other significant peaks, but substantially increased background noise.

EDS analysis was also carried out on the JEOL-733 system which was capable of higher accelerating voltages and better resolution for the lower x-ray energies. Resultant spectra from statoliths (Fig. 10) showed the strong P and Ca peaks, as well as weaker peaks for Mg, Cl, and K. Given the approximate concentration levels for trace elements as determined by ICP analysis (discussed below) and the detection limits for EDS, the only elements which would be expected to show in the EDS spectra are Ca, P, Mg and Na. Potassium and Cl are also probable, although not reliably measured with

the ICP. The Na x-ray energy peaks are too close to the lower spectrum cutoff to show in the EDS analysis.

WDS

The WDS system was used for a more detailed spatial analysis of the statoliths and for its higher sensitivity compared with EDS (Heinrich and Newbury, 1986). In general WDS has detection limits an order of magnitude lower than EDS. This corresponds to a range of 0.01 to 0.3% or 100 to 3000 ppm. Total spectral scans with WDS are much more time consuming than with EDS, since slow crystal rotations with three separate crystals are necessary. However this is a good method to search for the presence of trace elements in unknown samples. A scan for a St. Marys specimen did not reveal any elements not detected with EDS, but the Na and K peaks were verified as more distinct.

As noted above, BEI views of statolith sections revealed that there is significant heterogeneity in average atomic weight. Fig. 11 shows additional views of this phenomenon in Little Salmon Creek and St. Marys River specimens. A number of WDS line scans were done across the bright/dark boundary with the crystal tuned for Mg and Ca. This analysis indicated that relative Mg concentrations are higher in the "darker" areas. Fig. 12 shows a representative line scan for Mg. The possible interpretations of these results were discussed earlier.

XRF

Under the operating conditions employed in the study, elements with atomic numbers lower than 16 are not readily detectable. Approximate minimum detectable concentrations (Fig. 13) range from about 1 to 100 ppm, or 0.0001 to .01%. These are under optimal conditions; due to the small size, irregular geometry and high level of background noise for the statolith samples, these stated detection levels may be a least one or two orders of magnitude too high. Statoliths were analyzed as either embedded, ground and polished sections or as whole or intact specimens. Some analyses were not done in a vacuum, resulting in Ar peaks also being added to the background.

Fig. 14 shows spectra for a control blank Spectra Cup compared with Mylar supported samples of St. Marys statoliths. The statolith samples were either two statoliths or fourteen. The larger sample was run twice to test the

effects of rotating the cup and changing the position of the individual statoliths. The control spectrum showed peaks for Ar (atmospheric), Ca, Fe, Cu, Zn, and Pb. These elements were apparently present in the plastic cups used as sample holders. A run with only two statoliths shows a perceptible increase in Ca and perhaps a slight elevation of Zn. Comparison of the control with 14 statoliths shows a large increase in Ca and a smaller rise in Zn. Comparison of the two 14 statolith runs showed good reproducibility with no apparent differences. A similar set of spectra for whole statoliths is shown in Fig.15; the only difference is that the beam was not collimated for this series, giving slightly higher background levels in the higher energy range. The control blank cup showed the same peaks and Ca was detectable for only two statoliths. Comparison of larger samples from the St. Marys River with Cayuga Inlet showed higher Ca and Zn versus the control, but no differences between the two in relative peak height.

The final series of XRF spectra (Fig. 16) is for the Spurr's embedded samples. These were also in an evacuated chamber which eliminates the Ar peak. A control section of Spurr's (no statoliths) showed the standard Spectra Cup elements (Ca, Fe, Cu, Zn, and Pb) with the addition of Cl from the Spurr's medium. A 14 statolith sample had elevated Ca and Zn.

8 The results from the XRF analyses were consistent with the sensitivity of the technique in relation to the very low trace levels present in the statoliths. Background noise from the Spectra Cups can probably be eliminated by alternate methods of specimen mounting, however XRF may still be too insensitive to most trace elements to give useful results when limited to a sample size of two statoliths.

NAA

More than 30 runs were made on statolith materials and assorted blanks and background counts. Tables 2 and 3 give estimates of the detection limits of NAA systems under ideal conditions. The operating conditions for the statolith analyses differed from these standards, particularly because of the small volume of material analyzed. Nevertheless the published values may serve as an approximate guide, and are particularly useful in determining relative sensitivities for different elements. Figs. 17-19 illustrate some typical NAA results. Table 4 summarizes information on all of the 59 gamma energy peaks observed during the analyses. The occurrence of these isotopes

in the various samples, blanks and background and their changing intensity with natural decay presented a very complex picture. Table 5 distills this information into its simplest form, where the effects of background and "dirty" containers are sorted out. The first five columns give the isotopes found in background readings and in the various gelatin and polyethylene capsules used to hold specimens. The last five columns show the occurrence of isotopes in statoliths and otoliths counted in "clean" capsules. Unfortunately some procedural difficulties allowed only very limited counts for the St. Marys samples; and these were after much of the shorter half-life isotopes had decayed to non-detectable levels. Undoubtedly Ca, Na, and Cl and other elements were also present. The pooled Cayuga Inlet sample (150 ug) contained Ca, Cl, K, Mn, Mg, and Na. The Mn is perhaps most interesting since it occurs in trace amounts and NAA is extremely sensitive to its presence. The Mn was also of special interest since it allowed for a calibration to determine absolute concentration. This was made possible because the bone standard also contained Mn and the count rate could be related to the mass of the sample and the known concentration (32$\pm$5 ppm). Equations using the decay constant for Mn-56 were used to calculate expected count rates at the end of the irradiation period for both bone and statolith samples irradiated for 2 min. This yielded an estimate of the count rate expected for a known quantity of Mn (in the bone) and then a calculation of the amount of Mn in the unknown sample (statolith) from the measured count rate. The result indicated an approximate concentration of 60 ppm Mn in the Cayuga Inlet statoliths. This compares favorably with ICP estimates of 35 ppm Mn.

It should be remembered that pooled statolith samples were used and results for only two statoliths would show even less significance. NAA might be a viable technique if Mn is of paticular importance. The extremely low detection limit for Mn (0.001-0.010 ppb) by NAA exceeds the capabilities of all other analytical methods. Magnesium might also be of interest however the very short half-life of Mg presents additional analysis problems for routine inspections. Magnesium is also apparently much more abundant in the statoliths and may be best quantified by other techniques.

A significant advantage of NAA is that it is non-destructive and samples can be re-run for verification or analyzed by other techniques. Unfortunately the analyses to date have not revealed the presence of detectable levels of unusual trace elements. The small size of statoliths has been a limiting factor and the technique is being pushed to its practical limits.

ICP

Analysis by ICP proved to be the most informative of the methods applied during this study. Specimen preparation was fairly simple and data collection was rapid; yielding a direct reading in ppm of 17 elements. As long as no significant interferences were suspected, little additional data interpretation or manipulation was required. Thirteen samples were run, including pooled statolith samples, the bone standard, St. Marys water, and trout otoliths. The number of statoliths and masses for the lamprey samples were:

CODE	NO. STATOLITHS	MASS ug
STM1	15	95
STM2	2	13
STM3	9	57
CAY1	38	312
CAY2	38	312
LC	19	114
GR	20	136

The raw results for all of the ICP analyses are given in Table 6. Some of the values were below the detection limits and should be discounted. Values less than 10X the detection limits should still be considered suspect. Table 7 compares the concentrations measured (ug/ml or ppm) with the expected detection limits. For the statolith samples, P, Ca, Mg, Zn, and Na, were clearly detectable, with Mn, Fe, Al and Cr borderline. Similar comparisons can be made for the water, bone and otolith samples. Since the sample masses were highly variable, results were standardized to Ca to see differences in relative concentration (Table 8). This exercise also served to check that the derived site-specific otolith masses used to calculate absolute concentrations

did not introduce a bias. The lamprey samples are seen to be fairly consistent, especially when comparing the two Cayuga Inlet replicates. An obvious outlier is the Na level in STM1. This is explainable as an artifact where the ICP technician rinsed the gelatin capsule with water to flush statoliths into the quartz test tube. The gelatin has a very high sodium content and this probably contaminated the sample.

An estimate of the accuracy of the ICP results is gained by comparison of the bone analysis with the known composition (Table 9). Corrected values refer to adjustments made to compensate for interference from high calcium levels in the sample. There is very good agreement for Mn, Fe, Cu, and Zn. Larger errors were apparent for Co, Cr and Pb, but the values were still reasonably close.

Table 10 summarizes the % composition of all the statolith and otolith samples. As expected the phosphorus levels in the otoliths are much lower since their primary constituent is calcium carbonate instead of the calcium phosphate of the statoliths. Again there is quite good reproducibility in the two sets of values for otoliths from the same rainbow trout. Differences between the rainbow trout and the bull trout are relatively minor. A greater difference was expected since the parent watersheds were dissimilar. The rainbow trout came from the Madison River which receives some mineral-rich waters from the thermal springs of Yellowstone Park. In contrast, the bull trout was from Flathead Lake and probably spent several years in the Flathead or Swann system, both of which are known to be very low in mineral content. The bull trout was obviously an older fish and this variable may have to be considered when making such comparisons.

Table 11 and Figs. 20a-c illustrate possible differences between lamprey collected at different localities. The graphs are plotted at progressively smaller scales on the Y-axis to show values for the trace elements more clearly. It should be remembered that the very lowest values are too close to the detection limits to be reliable. Furthermore, the calculation of error bars or confidence limits was not possible with the small number of samples analyzed. STM2, with only two statoliths, has not been plotted due to lack of space, however it can be seen from the table that the values for this sample were in good agreement with the larger St. Marys samples. On the coarsest scale (Fig. 20a) the overall impression is that all the localities show similar composition. The high sodium content in STM1 has already been

explained as an artifact. On a finer scale (Fig. 20b), the locality specific consistency in Mg and Na is more obvious. The higher Na content of the Cayuga Inlet samples is reasonable considering the local salt deposits and relatively high conductivity of the water (pers. obs.). The finest scale (Fig. 20c) also shows slight suggestions for site-specific differences in Mn, Fe and perhaps Zn. The best that can be said at this point is that there are trace elements in the statoliths and that pooled samples appear to show some differences between localities. What can be expected when only one or two statoliths are analyzed at a time? This is the requirement to determine the home stream of individual lampreys.

Table 12 gives the calculated ug per statolith for the different elements detected. These data were then used to calculate the approximate concentrations (in ppm) expected when the statoliths are dissolved in either 1 ml or 30 ul (Table 13). The larger volume is the sample size used in the current work and the smaller volume is possible with the addition of a special attachment called Direct Injection Nebulizer. A smaller volume obviously gives more concentrated solutions and therefore a much better chance of detecting very low levels of certain elements. Table 14 compares expected ICP results with detection limits: for two statoliths; without and with the Direct Injection Nebulizer. The 1 ml volume would restrict usable values to four or possibly five elements, including Ca and P which are of only limited interest. Use of the nebulizer adds five or more elements which should give much greater possibilities for discriminating between localities. A ratio of approximately 10:1 was used as the criterion for including an element.

Water analyses on the two St. Marys samples showed only the expected low levels of dissolved substances. Only Ca, Mg, and Na were clearly above the detection limits, and others such as P, Fe, B, Zn, and Cd were borderline. The water from Station A had consistently higher values for all elements and this might be an indication of industrial input, but sediment samples and/or concentration of the dissolved substances would be a better approach to determining the concentration levels to which the lampreys are exposed. Table 15 summarizes the results and compares the St. Marys values to some published means for Lake Superior (Rossmann, 1986). The latter analyses were done with graphite furnace Atomic Absorption Spectroscopy (AA), a very sensitive technique (see Table 16) but much more time consuming and requiring special instrumentation and procedures for each element analyzed.

There are some new developments in ICP technology which will have a large impact on future studies. Cornell wil be receiving a new instrument (a JY 70P) this spring. This state-of-the-art model will offer better resolution, hhigher sensitivity (at least 10X), several convienient options for doing comparative studies, and it will add about a dozen more elements (Ba, Sc, Li, V, Ti, Sn, Si, Hg, S and others). It will also be accept the injection nebulizer which will boost sensitivity another order of magnitude higher. All of these improvements should make a significant difference in the our ability to analyze trace elements in lamprey statoliths.

Another recently developed instrument, the ICP-Mass Spectrometer (ICP-MS), is now available which will yield sensitivities comparable to AA (Faires, 1986). The ICP-MS can detect essentially all elements of interest (Fig. 21) and analyses them concurrently. As can be seen in Table 16, it is generally equal to or better than AA on selected common trace elements. The ICP-MS would appear to be the instrument of choice for continued work on statolith chemoprints.

CONCLUSIONS

SEM examination of sea lamprey statoliths revealed a microstructure indicative fusion of otoconia. The otoconia grow by lamellar addition of extremely fine increments (<0.04 um). BEI views of sectioned and polished statoconia indicated the presence areas of lower average atomic number near the periphery of the statoliths. These areas were found to be relatively Mg rich when examined with a WDS electron microprobe.

EDS and WDS microprobe analysis demonstrated the presence of Ca, P, Na, K, and Mg, but no differences between lampreys collected at different localities.

XRF analyses were difficult because of the small size of the statoconia and require modifications of the specimen holder for further work. Preliminary results showed the presence of Ca, Zn, and possibly Fe. Examination of only two statoliths can give measurable quantities of Zn.

NAA appears to be a workable for certain elements such as Mn. Additional statolith constituents that were detectable were Ca, Cl, K, Mg, and Na. The small sample size represented by two statoliths is a significant problem for detecting and quantifying elemental composition by NAA.

Improved ICPs and an advanced technique called ICP-Mass Spectrometry hold the greatest promise for yielding distinctive chemoprints from lamprey statoliths. Standard 1 ml samples from pooled statolith samples (several individuals) clearly demonstrated the presence of P, Ca, Mg, Mn, Fe, and Na. There are indications of reproducibility of results and consistent differences between localities. Modification of the instrumentation to allow for smaller sample volumes (30 ul) or implementation of the ICP-MS will substantially improve sensitivity.

As a general conclusion, it is apparent that the small size of the statoliths requires that analytical techniques must be pushed to their limits before comprehensive compositional data will be available for statoliths from individual lamprey. This feasibility study has demonstrated the presence of several elements of interest and indicates that even more sophisticated procedures will probably demonstrate locality-specific differences in statolith composition.

RECOMMENDATIONS

Although the present study is not conclusive in demonstrating locality-specific differences in statolith composition, there are sufficient positive indications to justify some continued work on the problem. At this point the most important task is to analyze some samples with the ICP-MS. Some preliminary examinations are already planned (see attached letter). Once these results are received, the statoliths will have been characterized by the most sensitive and practical method available.

Another technical improvement would be to use a Direct Injection Nebulizer in conjunction with an upgraded ICP. This will increase average effective sensitivity by about two orders of magnitude. With this technique it would be worthwhile to run a number of replicates to get a better idea of statistical confidence, internal (locality) consistency, and differences

between sites. This would offer a more conclusive answer to these questions than has been possible with the instruments currently available.

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TABLES

FIGURES

FIGURE 1 Relationship between fish length and statolith mass for Cayuga Inlet lampreys. (from Volk and Brothers, unpublished ms).

FIGURE 2 Collection site for water samples taken on 12 Dec 1986.

FIGURE 3 a. SEM (SEI) micrograph of sectioned, polished and acid-etched statolith. In this photo and all others the first number in the inset is the length of the scale bar in um

b. As above. Note the straight facets where the growing otoconia come into contact.

FIGURE 4 a. SEM view of central region of an "included" otoconium.

b. As above.
c. Magnified area in 4b. Note the very fine increments (<.04 um).

FIGURE 5 a. SEM (BEI) micrograph of sectioned and polished statoconia from Little Salmon Creek lampreys. The cracks are artifacts. Note the darker areas near the edges.

b. Enlarged view of above.

c. Higher magnification showing otoconial forms.

FIGURE 6 SEM (BEI) micrograph of mid-sagittal section of statolith from Cayuga Inlet lamprey.

FIGURE 7 a. SEM (SEI) of embedded, sectioned and polished bone standard. The central particle is metallic.

b. SEM (BEI) of area in 7a showing metallic particle with higher atomic number the surrounding bone material.

FIGURE 8 Typical EDS detectability limits for a 200 second analysis at 25 K

FIGURE 9 EDS spectra from AMR 1000A.

a. Spurr's epoxy; "bug" on chlorine peak.

b. Bone plus Spurr's; "bug" on chlorine; first peak is phosphorus; large double peak is calcium.

c. Bone; "bug" on phosphorus; large double peak is calcium.

d. St. Marys specimen.

e. St. Marys specimen.

f. Little Salmon Creek specimen.

FIGURE 10 EDS spectra from JEOL-733.

a. Spot sample in Mg "rich" area. Note that phosphorus peak is relatively higher than the calcium peak.

b. Spot sample in central region. Note that the calcium peak is higher than the phosphorus.

c. Spot sample in Mg "rich" area; same counting time as 11b; relative heights of Ca and P peaks are reversed.

FIGURE 11 a. SEM (BEI) view of Little Salmon Creek specimen. Note "darker" material at periphery.

b. As above for St. Marys River specimen. The round spots are damage caused by the electron beam. The line in the lower right is the location of the line scan in Fig. 12.

FIGURE 12 WDS line scan for Mg across the BEI brightness boundary. Higher Mg concentration on the right.

FIGURE 13 Minimum detectable concentration in geological material for XRF. The line for Mo anode, Mo filter is most appropriate for the conditions used in this study.

Figure 14 XRF spectra. Conditions: 50 KV, no vacuum, no collimation, 1-20 KeV range.

a. Control; blank Spectra cup

b. 2 statoliths; St. Marys River.

c. 14 statoliths; St. Marys River.

d. 14 statoliths; Cayuga Inlet.

FIGURE 15 XRF spectra. Conditions: 50 KV, no vacuum, collimated beam, 1-20 KeV range.

a. Control; blank Spectra cup.

b. 2 statoliths; St. Marys River.

c. 14 statoliths; St. Marys River.

d. Same specimens as 15c; rotated.

FIGURE 16 XRF spectra for Spurr's epoxy embedded specimens. Conditions: (a and b) 40 KV, (c and d) 25 KV, evacuated, no collimation, (a and b) 1-20 Kev range, (c and d) 1-10 Kev range.

a. Control; blank Spurr's epoxy. Note occurrence of chlorine peak.

b. 14 statoliths; St. Marys River.

c. Same as 16a.

d. Same as 16b.

FIGURE 17 NAA gamma energy spectrum. Cayuga Inlet specimens (150 ug). Irradiated for two minutes. Counted at T + 32 min. Live time - 10 min. Full spectrum. Log scale.

FIGURE 18 NAA gamma energy spectra. Same sample as in 17. Half spectra at later times.

a. T + 14 min. Live time - 10 min.

b. T + 1440 min. Live time - 1 hr 40 min. Note the decay in the Mn peak and disappearance of Mg. The K-42 appears in the background because of the long counting time.

FIGURE 19 NAA gamma energy spectra. Bone standard (4.4 mg). Irradiated for two minutes. Full spectra. Log scale.

a. T + 8 min. Live time - 15 min.

b. T + 140 min. Live time - 1 hr 50 min. Note loss of Al and decay of Mn and Ca.

FIGURE 20 Percent composition of statoliths. See text for abbreviations.

a. Full y-axis scale.

b. Scale reduced to 2%.

c. Scale reduced to 0.1%. Phosphorus and calcium not shown.

FIGURE 21 Detection limits in ppb for ICP-MS.

TABLE 1

Summary of the results obtained in Intercomparison Run A-3/1 on calcined animal bone

Elements and units		Co(ppm)	Cr(ppm)	Cu(ppm)	Fe(mg/g)	Mn(ppm)	Pb(ppm)	Zn(ppm)
No. of reported	individual determinations	38	30	38	58	40	19	67
	laboratory means	8	7	10	12	10	6	15
No. of accepted	individual determinations	31	30	29	53	31	18	63
	laboratory means	6	7	8	11	8	5	13
Overall mean of accepted laboratory means (recommended value)		0.46	683*)	6.8	1.52	32	6.8	183
Stand.dev. of the overall mean	absolute	0.16	241	2.3	0.36	5	1	12
	rel. %	35	35	34	24	16	15	6.5
Stand. error of the overall mean	absolute	0.06	91	0.8	0.11	2	0.5	4
	rel. %	13	13	12	7.2	6.2	7.3	2.1
Uncertainty limits of the overall mean (probability level 0.95)	± absolute	0.16	228	1.9	0.24	5	1.3	7
	± rel. %	35	33	28	16	16	19	3.8

*) The chromium level is about 100 times greater than that normally observed in this kind of material. The metal was probably introduced during the preparation.

Single-element interference-free detection limits for TNAA

TABLE 2

Sensitivity(a), 10^{-12} g	Elements
1–10	Mn, Rh, In, Eu, Dy
10–100	Ar, V, Co, I, Cs, Yb, Ir, Sm, Ho, Lu, Au
100–1000	F, Na, Mg, Al, Sc, Ti, Ga, Br, Ge, As, Sr, Pd, Ag, Sb, Te, Ba, La, Nd, Er, W, Re
10^3–10^4	Cl, Cr, Ni, Cu, Zn, Se, Ru, Cd, Sn, Ce, Pr, Gd, Tb, Tm, Hf, Pt, Th, U
10^4–10^5	K, Ca, Co, Rb, Y, Mo, Ta, Os, Hg
10^5–10^6	Zr, Nb
10^6–10^7	Si, S, Fe

(a) Assumptions: Sample irradiated 1 h in a neutron flux of 10^{13} n/cm^2·s and γ counted using a 50-cm^3 Ge(Li) detector for 2 h (or one half-life if 15 min < $T_{1/2}$ < 1 h). If $T_{1/2}$ < 15 min, cycle measurements conducted over a 2-h period, with timing of each cycle being (irradiation time) = (count time) = $T_{1/2}$. Detection is defined as 100 counts recorded in the full-energy peak, with the source placed 2 cm (0.8 in.) from the detector container.

TABLE 3

																	F – 1
	Na	Mg											Al	Si	P	S	Cl
β	5 X 10^{-3}	5 X 10^{-1}											1 X 10^{-1}	5 X 10^{-2}	5 X 10^{-1}	5	1 X 10^{-2}
γ	5 X 10^{-3}	5 X 10^{-1}											1 X 10^{-2}	500	–	200	1 X 10^{-1}
	K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br
β	5 X 10^{-2}	1.0	1 X 10^{-2}	5 X 10^{-1}	5 X 10^{-3}	–	5 X 10^{-5}	50	5 X 10^{-3}	5 · 10^{-2}	? X 10^{-3}	1 X 10^{-1}	5 X 10^{-3}	5 X 10^{-3}	1 X 10^{-3}	–	5 X 10^{-3}
γ	5 X 10^{-2}	5	5 X 10^{-2}	5 X 10^{-2}	1 X 10^{-3}	1	5 X 10^{-5}	200	1 X 10^{-1}	5 · 10^{-1}	? X 10^{-3}	1 X 10^{-1}	5 X 10^{-3}	5 X 10^{-2}	5 X 10^{-3}	5	5 X 10^{-3}
	Rb	Sr		Zr	Nb	Mo		Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I
β	5 X 10^{-2}	5 X 10^{-3}		1	5 X 10^{-3}	5 X 10^{-1}		1 · 10^{-2}	1 X 10^{-3}	5 · 10^{-4}	≡ X 10^{-3}	5 X 10^{-2}	5 X 10^{-6}	5 X 10^{-1}	5 X 10^{-3}	5 X 10^{-2}	5 X 10^{-3}
γ	5	5 X 10^{-3}		1	1	1 X 10^{-1}		5 · 10^{-2}	5 X 10^{-4}	5	≡ X 10^{-3}	5 X 10^{-1}	1 X 10^{-4}	5 X 10^{-1}	1 X 10^{-2}	5 X 10^{-2}	1 X 10^{-2}
	Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg		Pb	Bi		
β	5 X 10^{-1}	5 X 10^{-2}	1 X 10^{-3}	–	5 X 10^{-2}	1 X 10^{-3}	5 X 10^{-4}	5 · 10^{-2}	1 X 10^{-4}	5 X 10^{-2}	≡ X 10^{-4}	–		10	5 X 10^{-1}		
γ	5 X 10^{-1}	1 X 10^{-1}	5 X 10^{-3}	1	5 X 10^{-1}	5 X 10^{-3}	1 X 10^{-3}	–	1 X 10^{-3}	1 X 10^{-1}	≡ X 10^{-4}	1 X 10^{-2}		–	–		

	Ce	Pr	Nd		Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
β	1 X 10^{-1}	5 X 10^{-4}	1 X 10^{-1}		5 X 10^{-4}	5 X 10^{-6}	1 X 10^{-2}	≡ X 10^{-2}	1 X 10^{-6}	1 X 10^{-4}	1 X 10^{-3}	1 X 10^{-2}	1 X 10^{-3}	5 X 10^{-5}
γ	1 X 10^{-1}	5 X 10^{-2}	1 X 10^{-1}		5 X 10^{-3}	5 X 10^{-4}	5 X 10^{-2}	? X 10^{-1}	5 X 10^{-6}	1 X 10^{-4}	1 X 10^{-3}	1 X 10^{-1}	1 X 10^{-3}	5 X 10^{-5}

	Th		U											
β	5 X 10^{-2}		5 X 10^{-3}											
γ	5 X 10^{-2}		5 X 10^{-3}											

Estimated sensitivities of neutron activation methods. Upper numbers correspond to β sensitivities in micrograms; lower numbers to γ sensitivities in micrograms. In each case samples were irradiated for 1 hr or less in a thermal neutron flux of 1.8 × 10^{12} neutrons/cm^2/sec. (From: V. P. Guinn and H. R. Lukens, Jr., in *Trace Analysis: Physical Methods*, G. H. Morrison, Ed., p. 345, Wiley: New York, 1965. Reprinted by permission of John Wiley & Sons, Inc.)

TABLE 4

GAMMA ENERGY PEAKS OBSERVED DURING NEUTRON ACTIVATION

Energy keV	Isotope (atm wt)	Intensity	Half-life minutes	Half-life hours	Half-life days	Half-life years
160.0	Sc 47	100			3.43	
186.2	Ra 226		Ra 226 series			
238.6	Pb 212		Th 232 series			
264.1	Ta 182	22			115.1	
264.5	Ge 77	100		11.3		
264.6	Se 75	100			121	
320.0	Cr 51	100			27.8	
346.3	Pt 191	100	88			
388.5	Sr 87m	100		2.84		
388.2	I 126	100			13.1	
411.0	Eu 152	7				12.2
411.8	Au 198	100			2.7	
489.5	Ca 47-	8			4.7	
	Sc 47				3.43	
511.0	Annihilation peak					
554.3	Br 82	80		35.9		
558.1	Ge 77	18		11.3		
564.0	Sb 122	100			2.75	
569.3	Cs 134	35				2.07
616.4	Os 190m	100	10			
617.0	Br 82	100		35.9		
619.0	Br 82	50		35.9		
698.3	Br 82	33		35.9		
776.6	Br 82	100		35.9		
827.8	Br 82	30		35.9		
844.0	Mg 27	100	9.5			
846.9	Mn 56	100		2.6		
857.0	Na escape peak					
1014.1	Mg 27	40	9.5			
1043.9	Br 82	37		35.9		
1063.0	?					
1115.4	Zn 65	100			245	
1115.4	Ni 65	60		2.56		
1121.2	Ta 182	100			115.1	
1145.0	Cl escape peak					
1173.1	Co 60	100				5.24
1293.6	Ar 41	100	110			
1296.9	Ca 47-	90			4.7	
	Sc 47				3.43	
1317.2	Br 82	38		35.9		
1332.4	Co 60	100				5.24
1368.4	Na 24	90		15		
1407.5	Eu 152	90				12.2
1408.0	?					
1460.7	K 40	100				1.25*10^9
1474.7	Br 82	28		35.9		

1524.7	K 42	100		12.5
1642.0	Cl 38	100	37.29	
1656.0	Cl escape peak			
1731.0	Na double escape peak			
1764.0	?			
1778.9	Al 28	100	2.31	
1810.7	Mn 56	25		2.9
2061.0	Ca double escape peak			
2166.8	Cl 38	70	37.3	
2242.0	Na escape peak			
2572.0	Ca escape peak			
2753.6	Na 24	100		15
3048.0	Ca double escape peak			
3083.0	Ca 49	100	8.8	
3102.4	S 37	100	5.1	

TABLE 5

OCCURRENCE OF GAMMA ENERGY PEAKS

ISOTOPE (ATM WT)	BACKGROUND	GEL.CAP.	POLY.CAP. 1	POLY.CAP. 2	POLY.CAP. 3	STM	CAY	BONE	TROUT BULL	TROUT RAINBOW
Al 28								X	?	
Ar 41		X	X	X	X					
Br 82			X	X	X					
Ca 49							X	X	X	X
Cl 38		X	X	X	X		X	X	X	X
Co 60	X									
Cr 51								?	?	
Cs 134	X									
Eu 152								X		
K 40	X									
K 42			X				X	X		X
Mn 56		X	X			X	X	X	X	X
Mg 27							X	X		
Na 24		X	X	X	X		X	X	X	X
Pb 212	X									
Ra 226	X									
Sc 47									X	X
Sr 87									X	X
Zn 65	X									

TABLE 6

ICP RESULTS FOR 1ml ANALYSED (PPM)

ELEMENT	DET LMT ppm	STM1	STM2	STM3	CAY1	CAY2	LC	GR	BONE	WATER A	WATER B	RAINBOW A	RAINBOW B	BULL
P	0.0110	8.5900	1.5900	6.7700	30.3000	29.6000	12.5000	13.9000	541.0000	0.0450	0.0000	0.6818	0.7807	1.9540
Ca	0.0450	21.8000	3.5200	15.1000	68.5000	64.9000	27.0000	28.6000	1183.000	14.6000	14.1000	3799	3942	5991
Mg	0.0030	0.5770	0.0620	0.3070	1.4500	1.3500	0.6460	0.5940	22.3000	3.2700	3.1700	0.0569	0.0548	0.1251
Mn	0.0006	0.0030	0.0000	0.0020	0.0110	0.0100	0.0070	0.0060	0.0880	0.0000	0.0000	0.0722	0.0755	0.0018
Fe	0.0020	0.0120	0.0090	0.0130	0.0160	0.0040	0.0380	0.0860	4.6500	0.0080	0.0010	0.0000	0.0000	0.1694
Cu	0.0060	0.0010	0.0000	0.0030	0.0050	0.0000	0.0020	0.0030	0.0270	0.0060	0.0040	0.0015	0.0062	0.0085
B	0.0010	0.0000	0.0000	0.0020	0.0010	0.0000	0.0000	0.0000	0.0070	0.0060	0.0060	0.0000	0.0000	0.0000
Zn	0.0006	0.0150	0.0060	0.0190	0.0190	0.0120	0.0140	0.0060	0.5610	0.0020	0.0020	0.2051	0.2208	0.3944
Al	0.0020	0.0000	0.0060	0.0130	0.0150	0.0000	0.0220	0.0610	0.3030	0.0020	0.0080	0.0000	0.0000	0.0000
Na	0.0060	13.2000	0.0330	0.1820	4.8000	5.5500	0.3170	0.2520	36.8000	2.0200	1.4300	29.6568	31.4363	69.6433
Co	0.0020	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.0890	0.0000	0.0040	0.0000	0.0000	0.0000
Cd	0.0016	0.0000	0.0000	0.0040	0.0050	0.0040	0.0000	0.0000	0.0080	0.0080	0.0050	0.0000	0.0153	0.0131
Cr	0.0020	0.0000	0.0080	0.0080	0.0080	0.0000	0.0120	0.0019	0.5770	0.0020	0.0030	0.0065	0.0000	0.0010
Ni	0.0160	0.0000	0.0000	0.0050	0.0000	0.0000	0.0100	0.0000	0.3790	0.0080	0.0070	0.0000	0.0091	0.0000
Pb	0.0140	0.0000	0.0190	0.0000	0.0030	0.0000	0.0060	0.0000	0.0840	0.0000	0.0000	0.0000	0.0000	0.0000

TABLE 7

ICP RESULTS (ug/ml)/DETECTION LIMIT(ug/ml)

ELEMENT	STM1	STM2	STM3	CAY1	CAY2	LC	GR	BONE	WATER A	WATER B	RAINBOW A	RAINBOW B	BULL
P	781	145	615	2755	2691	1136	1264	49182	4	0	62	71	178
Ca	484	78	336	1522	1442	600	636	26289	324	313	84422	87600	133133
Mg	192	21	102	483	450	215	198	7433	1090	1057	19	18	42
Mn	5	0	3	18	17	12	10	147	0	0	120	126	3
Fe	6	5	7	8	2	19	43	2325	4	1	0	0	85
Cu	0	0	1	1	0	0	1	5	1	1	0	1	1
B	0	0	2	1	0	0	0	7	6	6	0	0	0
Zn	25	10	32	32	20	23	10	935	3	3	342	368	657
Al	0	3	7	8	0	11	31	152	1	4	0	0	0
Na	2200	6	30	800	925	53	42	6133	337	238	4943	5239	11607
Co	0	0	0	0	0	0	0	45	0	2	0	0	0
Cd	0	0	3	3	3	0	0	5	5	3	0	10	8
Cr	0	4	4	4	0	6	1	289	1	2	3	0	1
Ni	0	0	0	0	0	1	0	24	1	0	0	1	0
Pb	0	1	0	0	0	0	0	6	0	0	0	0	0

TABLE 8

ELEMENTAL COMPOSITION AS % OF CALCIUM

ELEMENT	DET LMT ug	STM1	STM2	STM3	CAY1	CAY2	LC	GR	BONE	WATER A	WATER B	RAINBOW A	RAINBOW B	BULL
P	0.0110	39.404	45.170	44.835	44.234	45.609	46.296	48.601	45.731	0.308	0.000	0.018	0.020	0.033
Ca	0.0450	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000
Mg	0.0030	2.647	1.761	2.033	2.117	2.080	2.393	2.077	1.885	22.397	22.482	0.001	0.001	0.002
Mn	0.0006	0.014	0.000	0.013	0.016	0.015	0.026	0.021	0.007	0.000	0.000	0.002	0.002	0.000
Fe	0.0020	0.055	0.256	0.086	0.023	0.006	0.141	0.301	0.393	0.055	0.007	0.000	0.000	0.003
Cu	0.0060	0.005	0.000	0.020	0.007	0.000	0.007	0.010	0.002	0.041	0.028	0.000	0.000	0.000
B	0.0010	0.000	0.000	0.013	0.001	0.000	0.000	0.000	0.001	0.041	0.043	0.000	0.000	0.000
Zn	0.0006	0.069	0.170	0.126	0.028	0.018	0.052	0.021	0.047	0.014	0.014	0.005	0.006	0.007
Al	0.0020	0.000	0.170	0.086	0.022	0.000	0.081	0.213	0.026	0.014	0.057	0.000	0.000	0.000
Na	0.0060	60.550	0.938	1.205	7.007	8.552	1.174	0.881	3.111	13.836	10.142	0.781	0.797	1.162
Co	0.0020	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.008	0.000	0.028	0.000	0.000	0.000
Cd	0.0016	0.000	0.000	0.027	0.007	0.006	0.000	0.000	0.001	0.055	0.035	0.000	0.000	0.000
Cr	0.0020	0.000	0.227	0.053	0.012	0.000	0.044	0.007	0.049	0.014	0.021	0.000	0.000	0.000
Ni	0.0160	0.000	0.000	0.033	0.000	0.000	0.037	0.000	0.032	0.055	0.050	0.000	0.000	0.000
Pb	0.0140	0.000	0.540	0.000	0.004	0.000	0.022	0.000	0.007	0.000	0.000	0.000	0.000	0.000

TABLE 9

BONE SAMPLE, STANDARD

ELEMENT	UNCORRECTED ppm	CORRECTED ppm	KNOWN mean ppm	95% confid. + or -	UNCORRECTED % of Ca	CORRECTED % of Ca
P	174516.13	174414.00			45.7312	37.2100
Ca	381612.90	468710.00			100.0000	100.0000
Mg	7193.55	7187.00			1.8850	1.5300
Mn	28.39	21.82	32	5	0.0074	0.0047
Fe	1500.00	1487.00	1520	240	0.3931	0.3173
Cu	8.71	4.21	6.8	1.9	0.0023	0.0009
B	2.26	0.00			0.0006	0.0000
Zn	180.97	179.70	183	7	0.0474	0.0383
Al	97.74	27.60			0.0256	0.0059
Na	11870.97	11804.00			3.1107	2.5200
Co	28.71	11.20	0.46	0.16	0.0075	0.0024
Cd	2.58	0.00			0.0007	0.0000
Cr	186.13	176.20	683	228	0.0488	0.0376
Ni	122.26	116.90			0.0320	0.0249
Pb	27.10	0.00	6.8	1.3	0.0071	0.0000

TABLE 10

CONCENTRATION IN STATOLITHS AND OTOLITHS (%)

ELEMENT	STM1	STM2	STM3	CAY1	CAY2	LC	GR	RAINBOW A	RAINBOW B	BULL
P	9.0899	12.6190	12.3091	9.7240	9.4994	9.6749	11.5833	0.0068	0.0084	0.0116
Ca	23.0688	27.9365	27.4545	21.9833	20.8280	20.8978	23.8333	37.9900	42.3871	35.4497
Mg	0.6106	0.4921	0.5582	0.4653	0.4332	0.5000	0.4950	0.0006	0.0006	0.0007
Mn	0.0032	0.0000	0.0036	0.0035	0.0032	0.0054	0.0050	0.0007	0.0008	0.0000
Fe	0.0127	0.0714	0.0236	0.0051	0.0013	0.0294	0.0717	0.0000	0.0000	0.0010
Cu	0.0011	0.0000	0.0055	0.0016	0.0000	0.0015	0.0025	0.0000	0.0001	0.0001
B	0.0000	0.0000	0.0036	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Zn	0.0159	0.0476	0.0345	0.0061	0.0039	0.0108	0.0050	0.0021	0.0024	0.0023
Al	0.0000	0.0476	0.0236	0.0048	0.0000	0.0170	0.0508	0.0000	0.0000	0.0000
Na	13.9683	0.2619	0.3309	1.5404	1.7811	0.2454	0.2100	0.2966	0.3380	0.4121
Co	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000
Cd	0.0000	0.0000	0.0073	0.0016	0.0013	0.0000	0.0000	0.0000	0.0002	0.0001
Cr	0.0000	0.0635	0.0145	0.0026	0.0000	0.0093	0.0016	0.0001	0.0000	0.0000
Ni	0.0000	0.0000	0.0091	0.0000	0.0000	0.0077	0.0000	0.0000	0.0001	0.0000
Pb	0.0000	0.1508	0.0000	0.0010	0.0000	0.0046	0.0000	0.0000	0.0000	0.0000

TABLE 11

CONCENTRATION IN STATOLITHS (PPM)

ELEMENT	DET LMT ppm	STM1	STM2	STM3	CAY1	CAY2	LC	GR
P	0.0110	90899.5	126190.5	119400.4	97240.1	94993.6	96749.2	115833.3
Ca	0.0450	230687.8	279365.1	266313.9	219833.1	208279.8	208978.3	238333.3
Mg	0.0030	6105.8	4920.6	5414.5	4653.4	4332.5	5000.0	4950.0
Mn	0.0006	31.7	0.0	35.3	35.3	32.1	54.2	50.0
Fe	0.0020	127.0	714.3	229.3	51.3	12.8	294.1	716.7
Cu	0.0060	10.6	0.0	52.9	16.0	0.0	15.5	25.0
B	0.0010	0.0	0.0	35.3	3.2	0.0	0.0	0.0
Zn	0.0006	158.7	476.2	335.1	61.0	38.5	108.4	50.0
Al	0.0020	0.0	476.2	229.3	48.1	0.0	170.3	508.3
Na	0.0060	139682.5	2619.0	3209.9	15404.4	17811.3	2453.6	2100.0
Co	0.0020	0.0	0.0	0.0	0.0	0.0	0.0	1.7
Cd	0.0016	0.0	0.0	70.5	16.0	12.8	0.0	0.0
Cr	0.0020	0.0	634.9	141.1	25.7	0.0	92.9	15.8
Ni	0.0160	0.0	0.0	88.2	0.0	0.0	77.4	0.0
Pb	0.0140	0.0	1507.9	0.0	9.6	0.0	46.4	0.0

TABLE 12

ug PER STATOLITH

ELEMENT	DET LMT ppm	STM1	STM2	STM3	CAY1	CAY2	LC	GR
P	0.0110	0.5727	0.7950	0.7522	0.7974	0.7789	0.6579	0.6950
Ca	0.0450	1.4533	1.7600	1.6778	1.8026	1.7079	1.4211	1.4300
Mg	0.0030	0.0385	0.0310	0.0341	0.0382	0.0355	0.0340	0.0297
Mn	0.0006	0.0002	0.0000	0.0002	0.0003	0.0003	0.0004	0.0003
Fe	0.0020	0.0008	0.0045	0.0014	0.0004	0.0001	0.0020	0.0043
Cu	0.0060	0.0001	0.0000	0.0003	0.0001	0.0000	0.0001	0.0002
B	0.0010	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000
Zn	0.0006	0.0010	0.0030	0.0021	0.0005	0.0003	0.0007	0.0003
Al	0.0020	0.0000	0.0030	0.0014	0.0004	0.0000	0.0012	0.0031
Na	0.0060	0.8800	0.0165	0.0202	0.1263	0.1461	0.0167	0.0126
Co	0.0020	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Cd	0.0016	0.0000	0.0000	0.0004	0.0001	0.0001	0.0000	0.0000
Cr	0.0020	0.0000	0.0040	0.0009	0.0002	0.0000	0.0006	0.0001
Ni	0.0160	0.0000	0.0000	0.0006	0.0000	0.0000	0.0005	0.0000
Pb	0.0140	0.0000	0.0095	0.0000	0.0001	0.0000	0.0003	0.0000

TABLE 13

ppm EXPECTED FOR 2 STATOLITHS IN A 1ml SAMPLE

ELEMENT	DET LMT ppm	STM1	STM2	STM3	CAY1	CAY2	LC	GR
P	0.0110	1.1453	1.5700	1.5044	1.5947	1.5579	1.3158	1.3900
Ca	0.0450	2.9067	3.5200	3.3556	3.6053	3.4158	2.8421	2.8600
Mg	0.0030	0.0769	0.0620	0.0682	0.0763	0.0711	0.0680	0.0594
Mn	0.0006	0.0004	0.0000	0.0004	0.0006	0.0005	0.0007	0.0006
Fe	0.0020	0.0016	0.0090	0.0029	0.0008	0.0002	0.0040	0.0086
Cu	0.0060	0.0001	0.0000	0.0007	0.0003	0.0000	0.0002	0.0003
B	0.0010	0.0000	0.0000	0.0004	0.0001	0.0000	0.0000	0.0000
Zn	0.0006	0.0020	0.0060	0.0042	0.0010	0.0006	0.0015	0.0006
Al	0.0020	0.0000	0.0060	0.0029	0.0008	0.0000	0.0023	0.0061
Na	0.0060	1.7600	0.0330	0.0404	0.2526	0.2921	0.0334	0.0252
Co	0.0020	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Cd	0.0016	0.0000	0.0000	0.0009	0.0003	0.0002	0.0000	0.0000
Cr	0.0020	0.0000	0.0080	0.0018	0.0004	0.0000	0.0013	0.0002
Ni	0.0160	0.0000	0.0000	0.0011	0.0000	0.0000	0.0011	0.0000
Pb	0.0140	0.0000	0.0190	0.0000	0.0002	0.0000	0.0006	0.0000

ppm EXPECTED FOR 2 STATOLITHS IN A 30ul SAMPLE

ELEMENT	DET LMT ppm	STM1	STM2	STM3	CAY1	CAY2	LC	GR
P	0.0110	38.1778	53.0000	50.1481	53.1579	51.9298	43.8596	46.3333
Ca	0.0450	96.8889	117.3333	111.8519	120.1754	113.8596	94.7368	95.3333
Mg	0.0030	2.5644	2.0667	2.2741	2.5439	2.3684	2.2667	1.9800
Mn	0.0006	0.0133	0.0000	0.0148	0.0193	0.0175	0.0246	0.0200
Fe	0.0020	0.0533	0.3000	0.0963	0.0281	0.0070	0.1333	0.2867
Cu	0.0060	0.0044	0.0000	0.0222	0.0088	0.0000	0.0070	0.0100
B	0.0010	0.0000	0.0000	0.0148	0.0018	0.0000	0.0000	0.0000
Zn	0.0006	0.0667	0.2000	0.1407	0.0333	0.0211	0.0491	0.0200
Al	0.0020	0.0000	0.2000	0.0963	0.0263	0.0000	0.0772	0.2033
Na	0.0060	58.6667	1.1000	1.3481	8.4211	9.7368	1.1123	0.8400
Co	0.0020	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0007
Cd	0.0016	0.0000	0.0000	0.0296	0.0088	0.0070	0.0000	0.0000
Cr	0.0020	0.0000	0.2667	0.0593	0.0140	0.0000	0.0421	0.0063
Ni	0.0160	0.0000	0.0000	0.0370	0.0000	0.0000	0.0351	0.0000
Pb	0.0140	0.0000	0.6333	0.0000	0.0053	0.0000	0.0211	0.0000

TABLE 14

EXPECTED ICP RESULTS (ppm)/DETECTION LIMIT (ppm)
2 STATOLITHS in 1ml

ELEMENT	STM1	STM2	STM3	CAY1	CAY2	LC	GR
P	104	145	137	145	142	120	126
Ca	65	78	75	80	76	63	64
Mg	26	21	23	25	24	23	20
Mn	1	0	1	1	1	1	1
Fe	1	5	1	0	0	2	4
Cu	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0
Zn	3	10	7	2	1	2	1
Al	0	3	1	0	0	1	3
Na	293	6	7	42	49	6	4
Co	0	0	0	0	0	0	0
Cd	0	0	1	0	0	0	0
Cr	0	4	1	0	0	1	0
Ni	0	0	0	0	0	0	0
Pb	0	1	0	0	0	0	0

EXPECTED ICP RESULTS (ppm)/DETECTION LIMIT (ppm)
2 STATOLITHS in 30ul

ELEMENT	STM1	STM2	STM3	CAY1	CAY2	LC	GR
P	3471	4818	4559	4833	4721	3987	4212
Ca	2153	2607	2486	2671	2530	2105	2119
Mg	855	689	758	848	789	756	660
Mn	22	0	25	32	29	41	33
Fe	27	150	48	14	4	67	143
Cu	1	0	4	1	0	1	2
B	0	0	15	2	0	0	0
Zn	111	333	235	56	35	82	33
Al	0	100	48	13	0	39	102
Na	9778	183	225	1404	1623	185	140
Co	0	0	0	0	0	0	0
Cd	0	0	19	5	4	0	0
Cr	0	133	30	7	0	21	3
Ni	0	0	2	0	0	2	0
Pb	0	45	0	0	0	2	0

TABLE 15

WATER ANALYSES
TRACE ELEMENT CONCENTRATIONS, ppb

ELEMENT	DET LMT ICP	WATER A	WATER B	SUPERIOR 1983	SUPERIOR HIST.MAX.	DET CRITERION AA
P	11	45	0			
Ca	45	14600	14100			
Mg	3	3270	3170			
Mn	0.6	0	0	.027-1	9	0.01
Fe	2	8	1	1-37	230	0.35
Cu	6	6	4	.68-2	230	0.11
B	1	6	6	0-24	24	51
Zn	0.6	2	2	.3-1.4	80	0.14
Al	2	2	8	1-24	24	0.65
Na	6	2020	1430			
Co	2	0	4	.00041-.0	1	0.012
Cd	1.6	8	5	.044-.07	0.25	0.0076
Cr	2	2	3	.012-.91	18	0.043
Ni	16	8	7	.018-.24	9	0.029
Pb	14	0	0	0-.13	7	0.055

TABLE 16

COMPARISON OF DETECTION LIMITS OF ICP, ICP - MASS SPECT. AND GRAPHITE FURNACE AA

	A	B	C	A/B	C/B
	ICP DET LMT	ICP-MASS SPECT. DET LMT	AA DET LMT		
ELEMENT	ppb	ppb	ppb		
K	1450.00	1000		1	
P	11.00	2000		0.0055	
Ca	45.00	5		9	
Mg	3.00	0.1		30	
Mn	0.60	0.04	0.01	15	0.25
Fe	2.00	0.2	0.35	10	1.75
Cu	6.00	0.03	0.11	200	3.67
B	1.00	0.08	51	13	637.50
Zn	0.60	0.08	0.14	8	1.75
Al	2.00	0.1	0.65	20	6.50
Na	6.00	0.06		100	
Co	2.00	0.01	0.012	200	1.20
Cd	1.60	0.07	0.0076	23	0.11
Cr	2.00	0.02	0.043	100	2.15
Ni	16.00	0.03	0.029	533	0.97
Pb	14.00	0.02	0.055	700	2.75

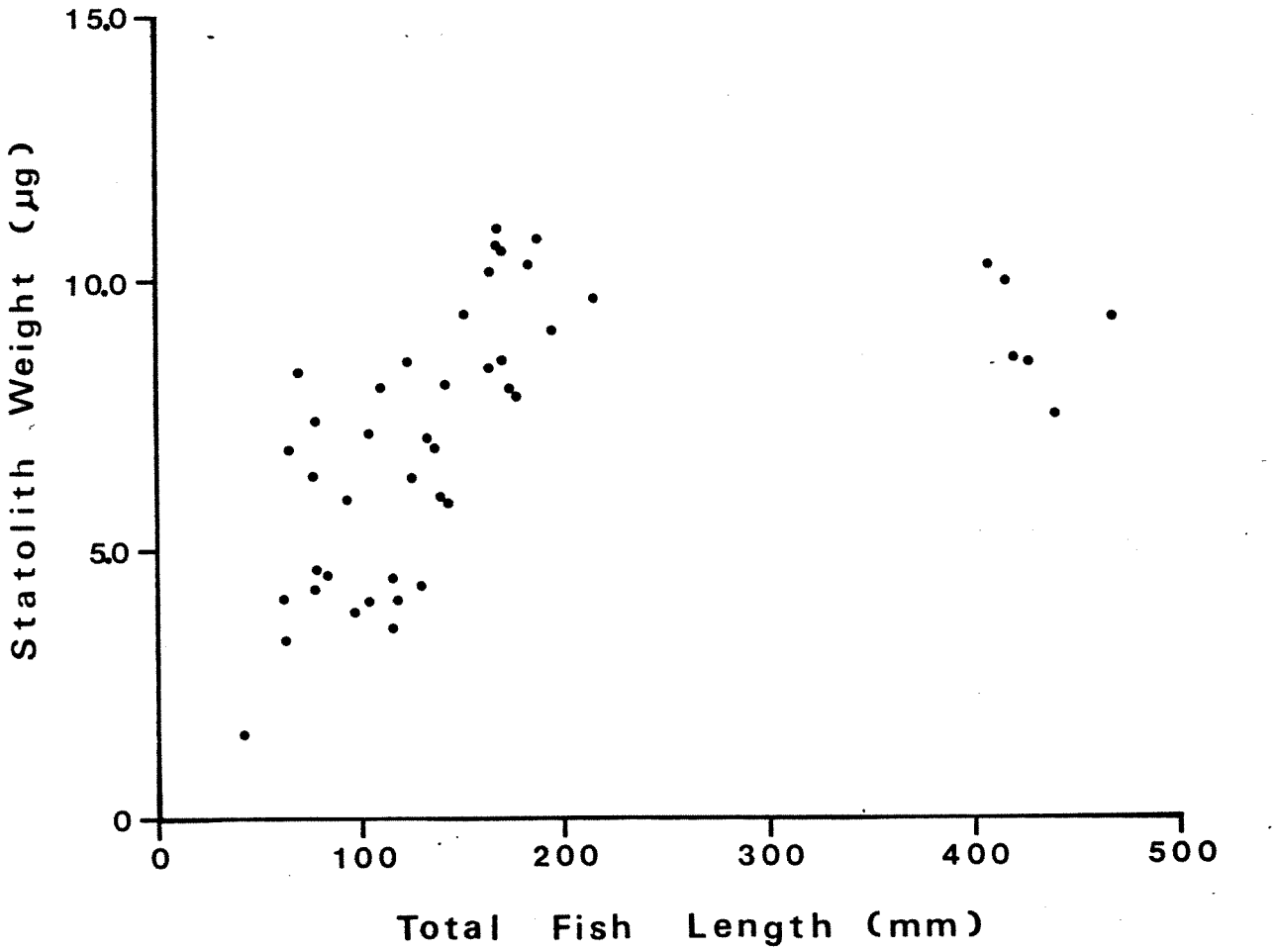

15.0
10.0
5.0
0
Statolith Weight (µg)
0
100
200
300
400
500
Total Fish Length (mm)

FIGURE 1

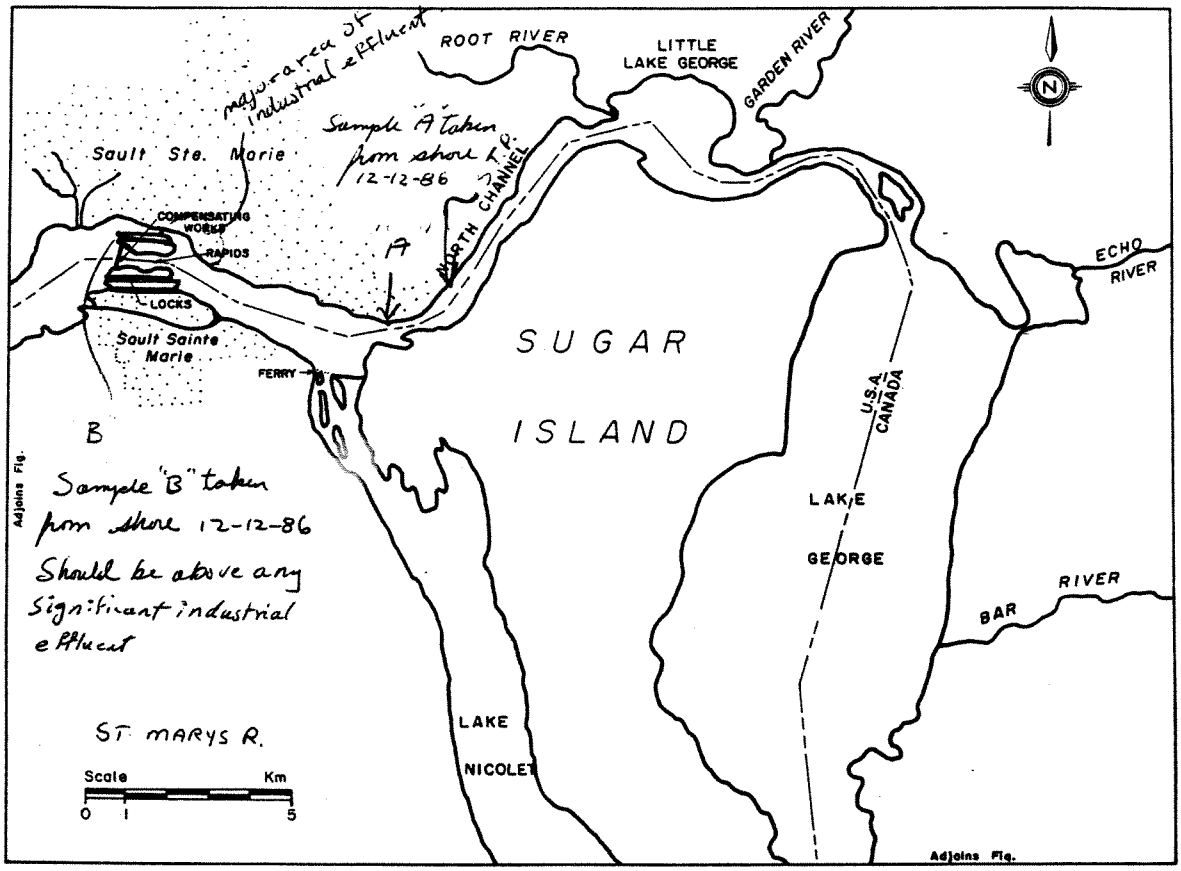
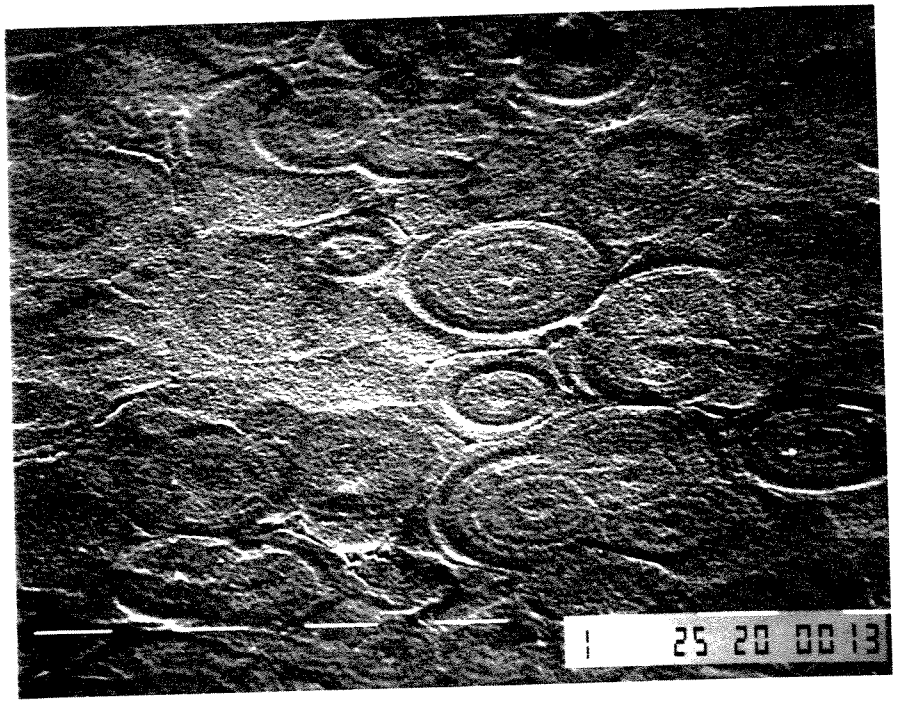
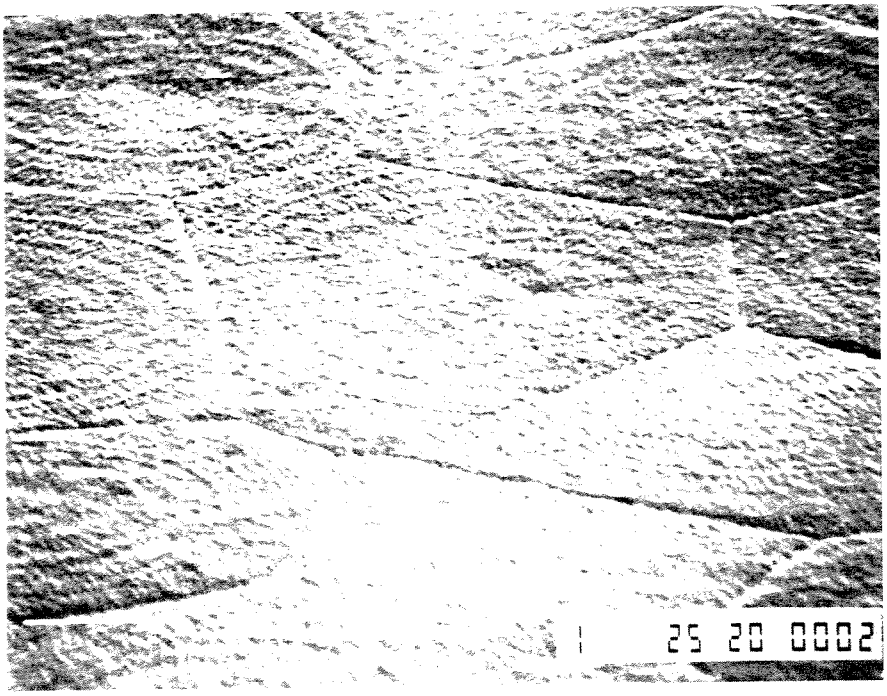

major area of industrial effluent
ROOT RIVER
LITTLE LAKE GEORGE
GARDEN RIVER
Sault Ste. Marie
Sample "A" taken from shore 12-12-86
NORTH CHANNEL
COMPENSATING WORKS
RAPIDS
LOCKS
Sault Sainte Marie
FERRY
A
B
SUGAR ISLAND
ECHO RIVER
U.S.A. CANADA
LAKE GEORGE
BAR RIVER
LAKE NICOLET
Sample "B" taken from shore 12-12-86
Should be above any significant industrial effluent
ST. MARYS R.
Scale
Km
0 1 5
Adjoins Fig.
Adjoins Fig.
N

FIGURE 2

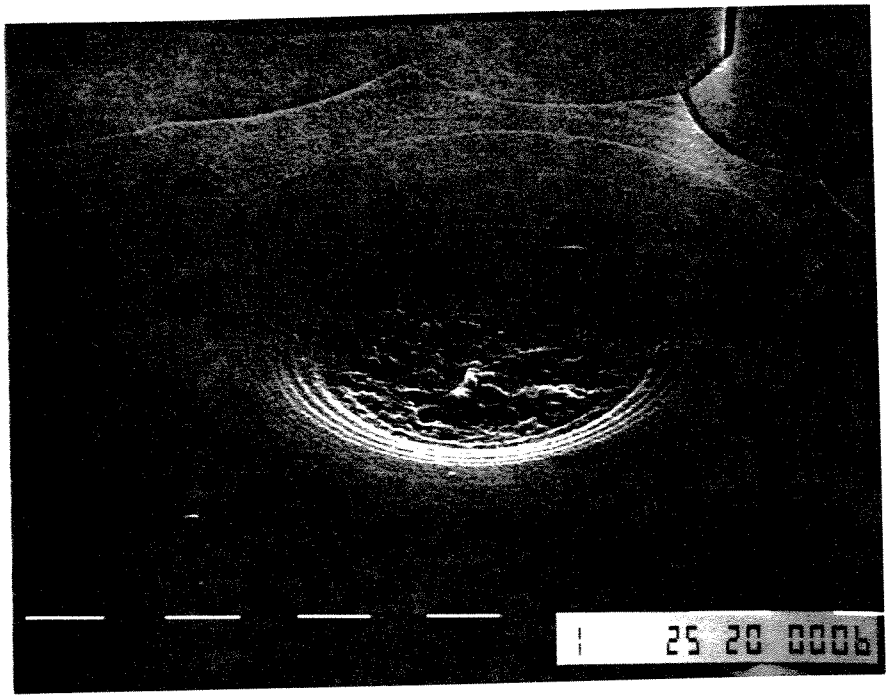


A

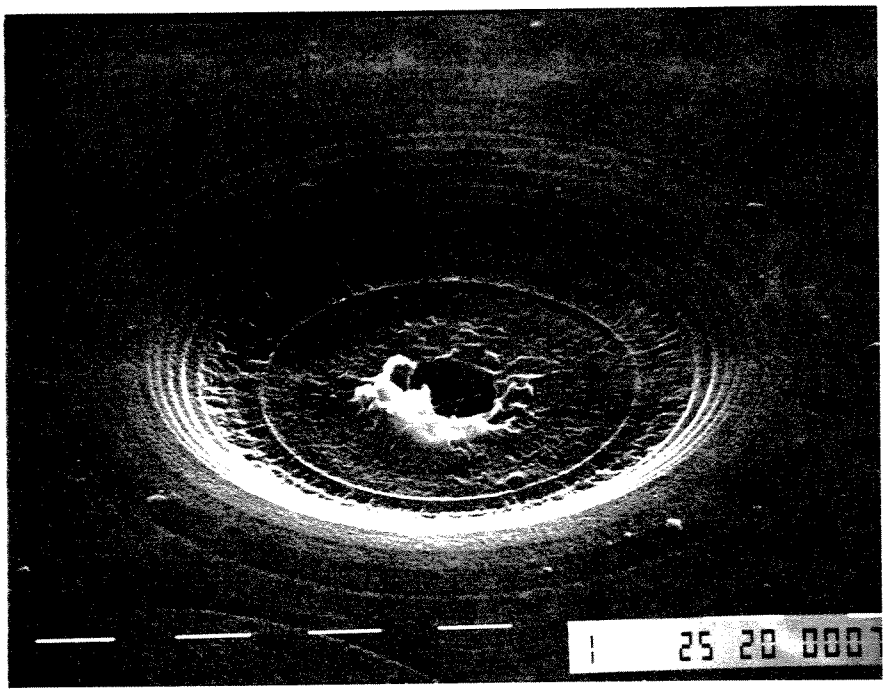


B

FIGURE 3

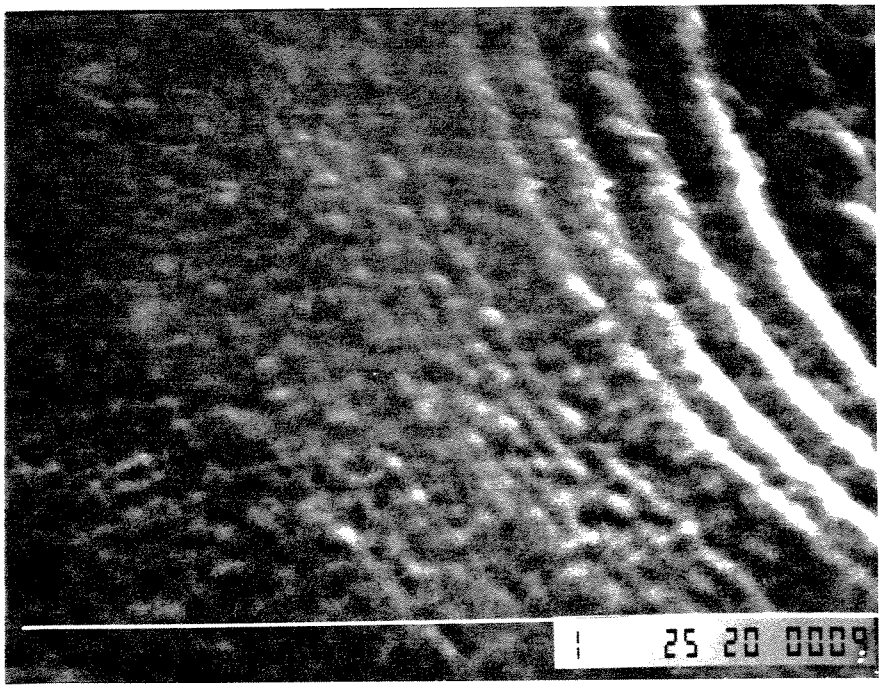


A

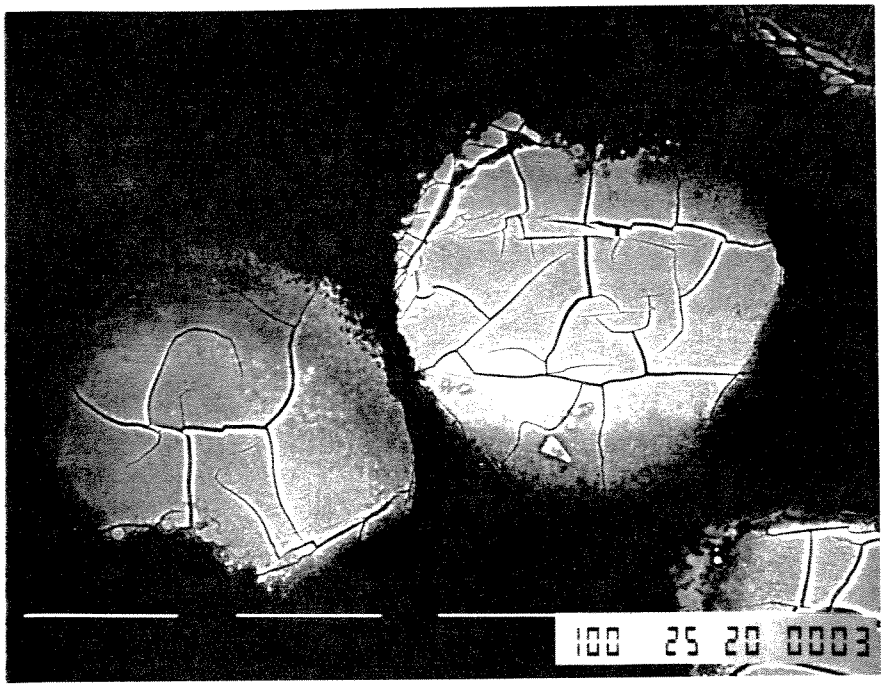


B

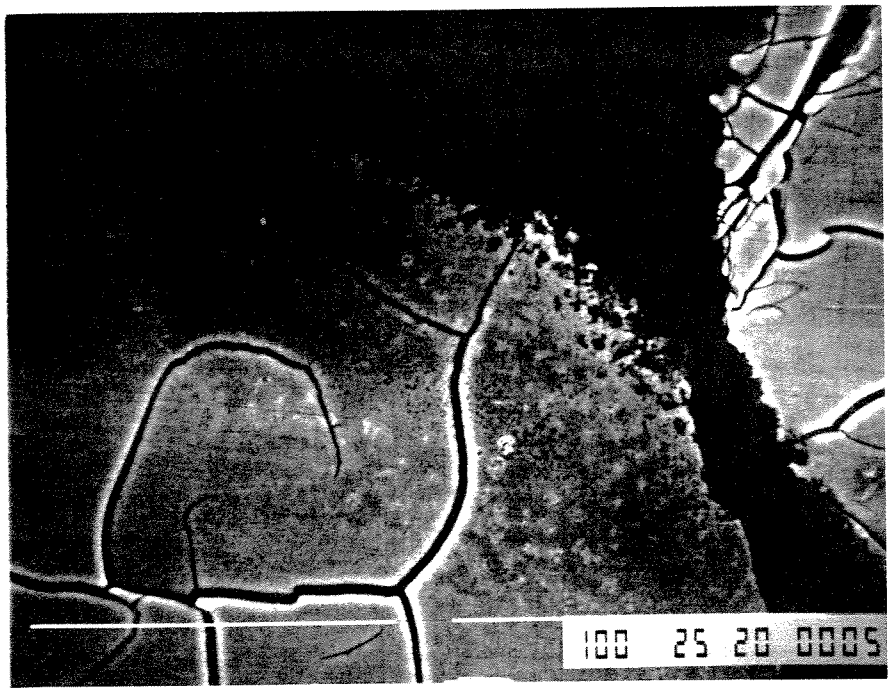
FIGURE 4



C

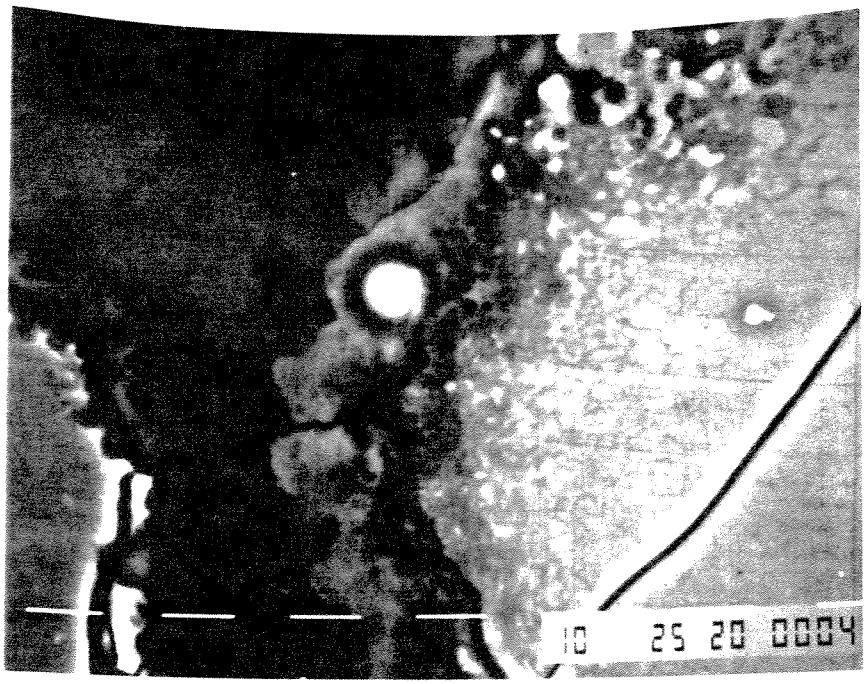


A



B

FIGURE 5



C

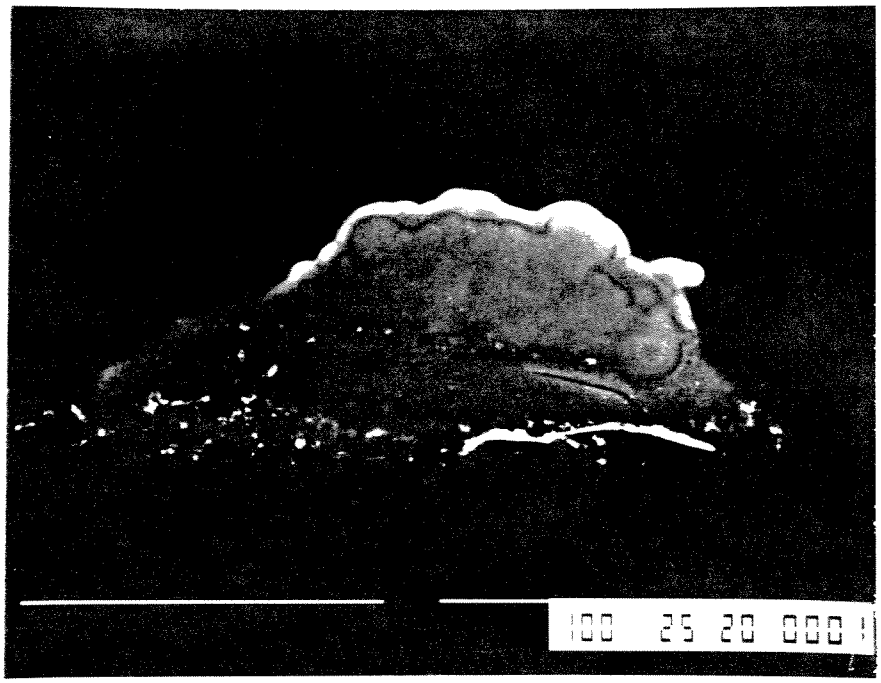
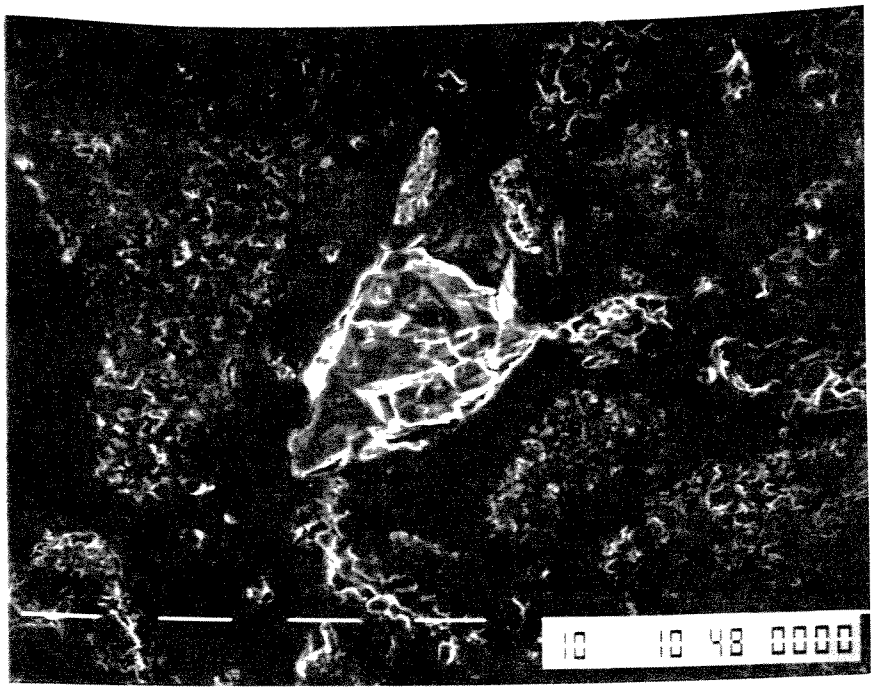
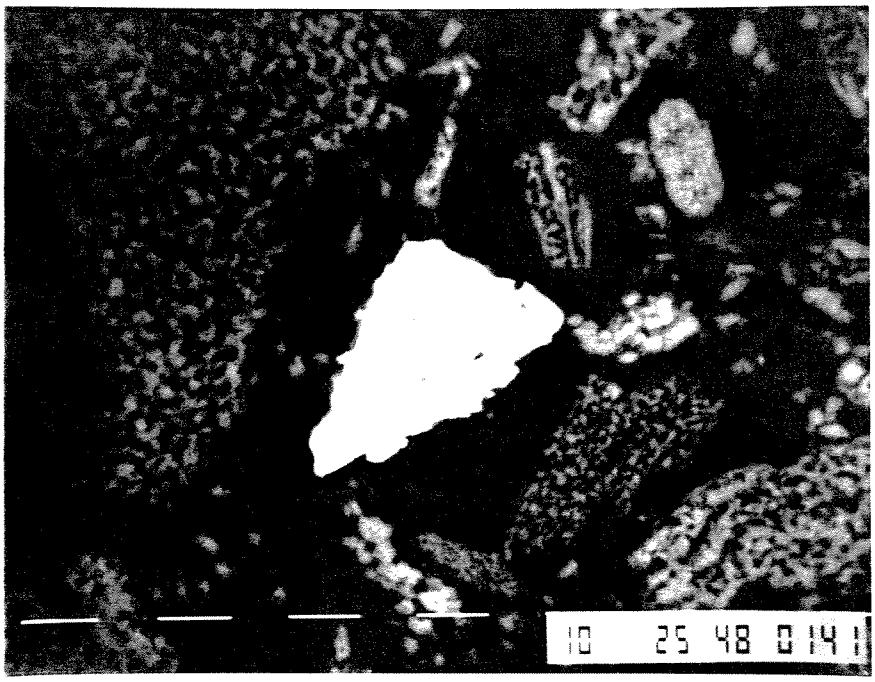


FIGURE 6



A



B

FIGURE 7

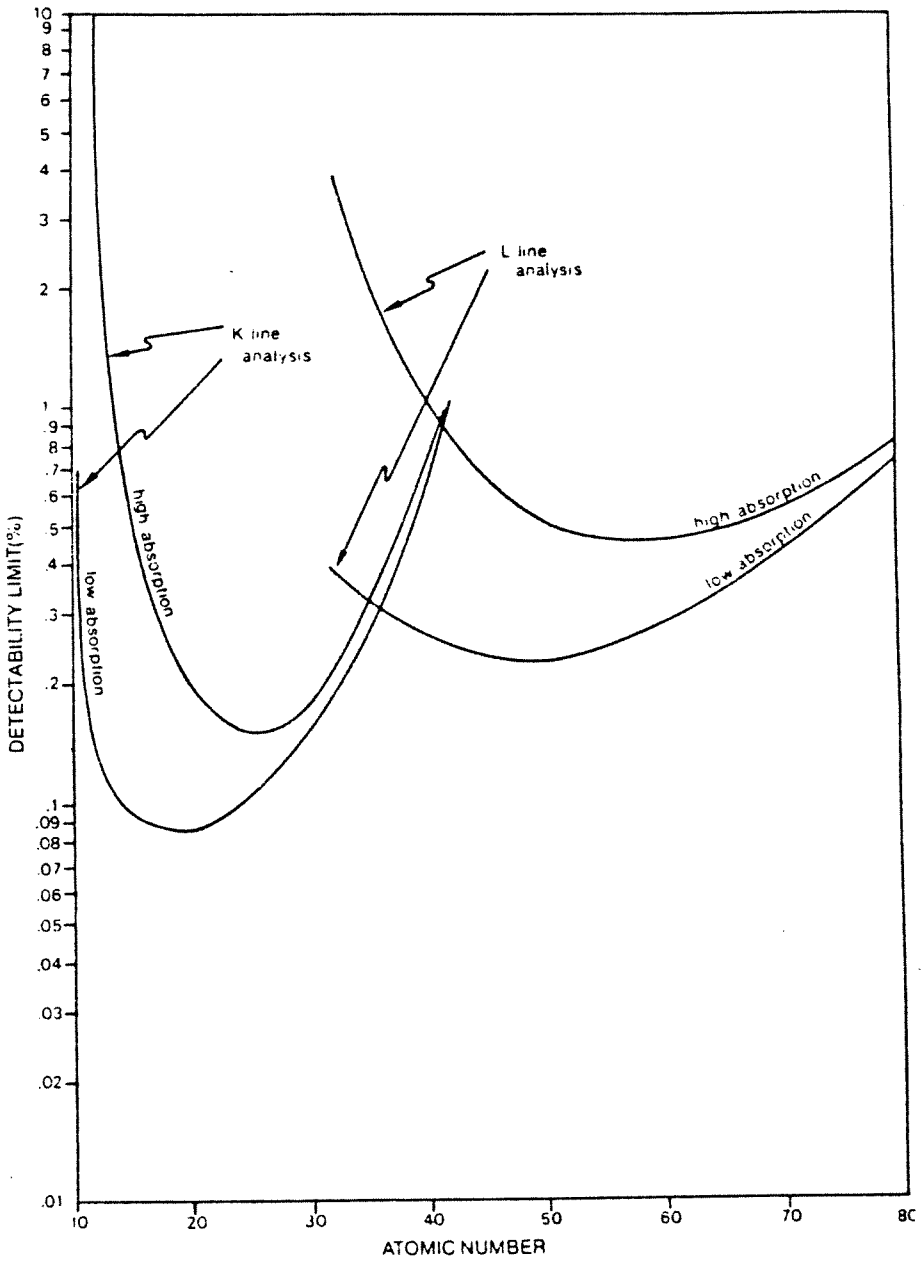
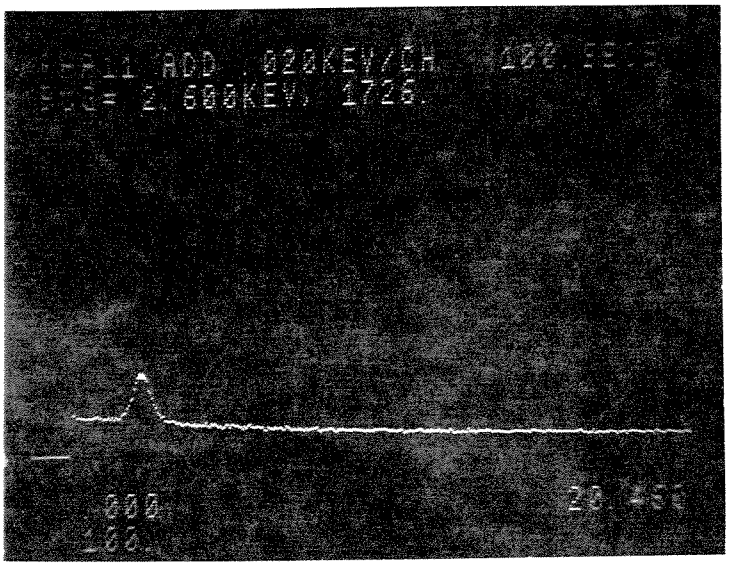
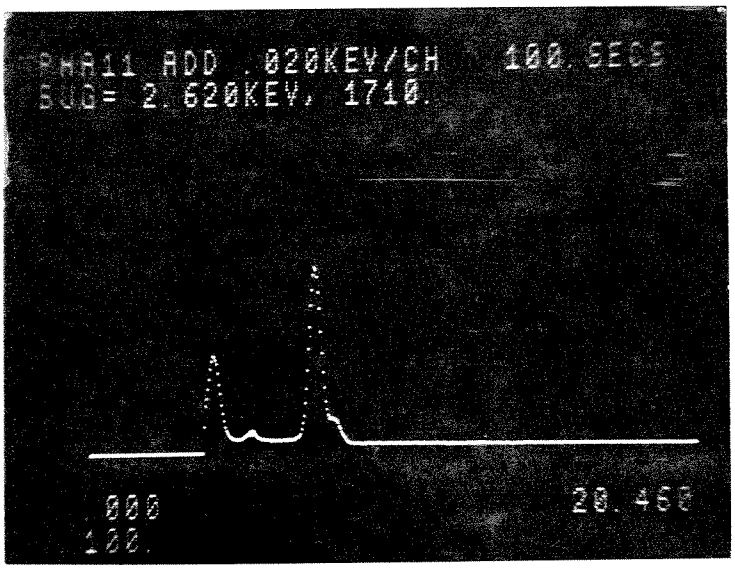

DETECTABILITY LIMIT(%)
ATOMIC NUMBER
K line analysis
L line analysis
high absorption
low absorption
high absorption
low absorption

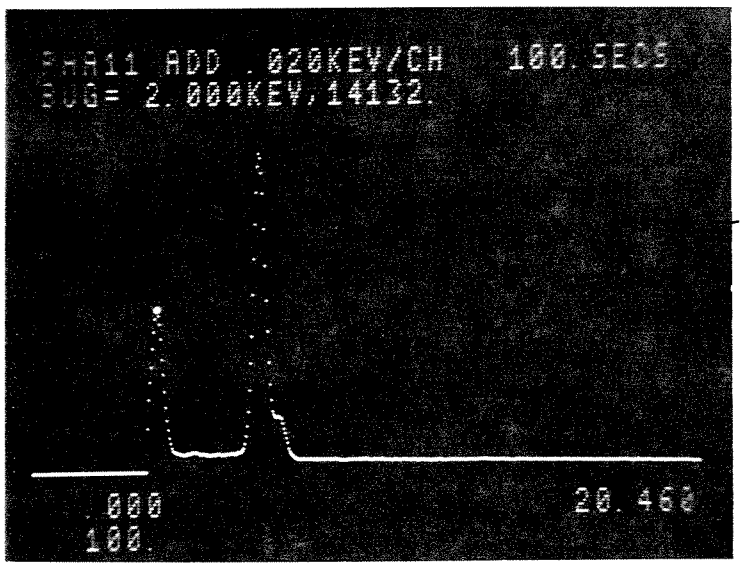
FIGURE 8


PHA11 ADD .020KEV/CH 100.SECS
SUG= 2.600KEV, 1726.
.000
100.
20.460

A

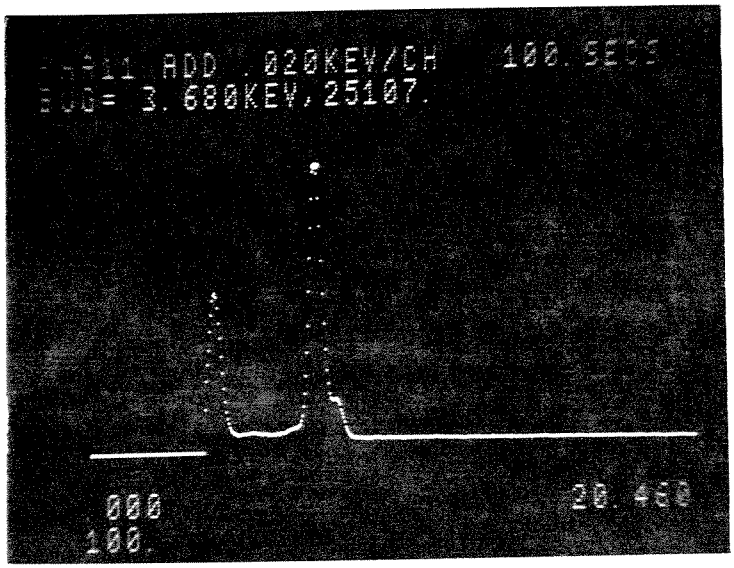

PHA11 ADD .020KEV/CH 100.SECS
SUG= 2.620KEV, 1710.
.000
100.
20.460

B

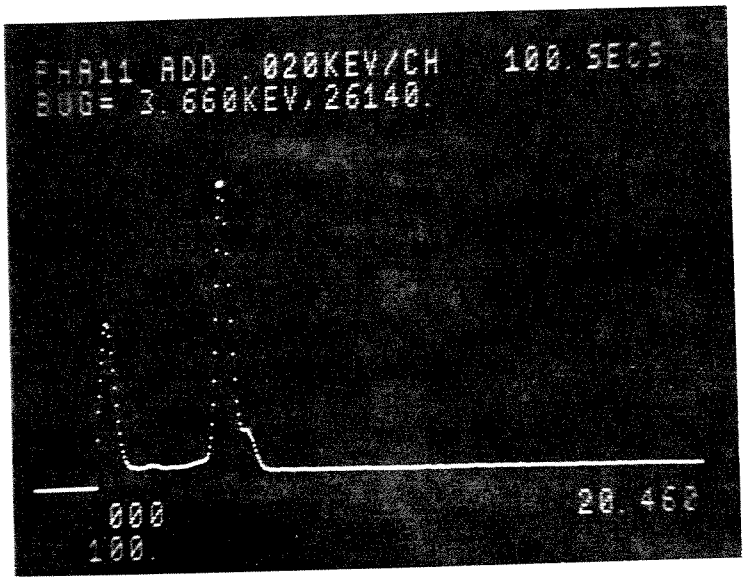

PHA11 ADD .020KEV/CH 100.SECS
SUG= 2.000KEV,14132.
.000
100.
20.460

C

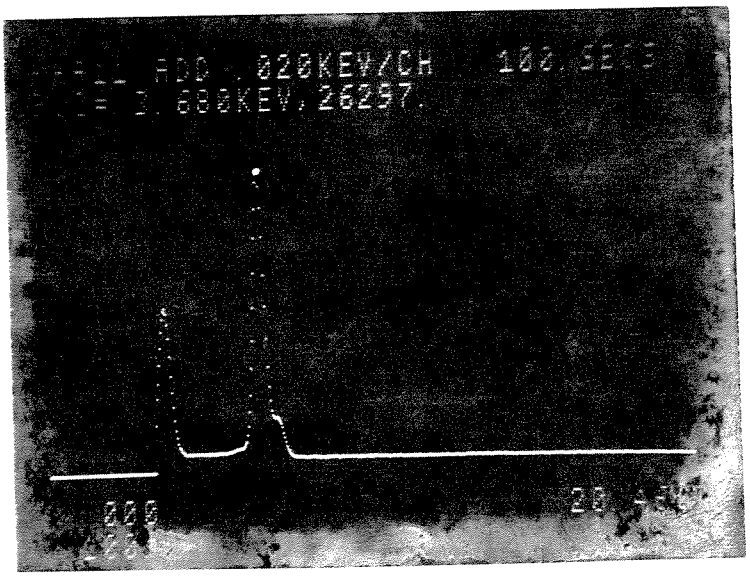
FIGURE 9


F-A11 ADD .020KEV/CH 100.SECS
BUG= 3.680KEV,25107.
000
100.
20.460

D


F-A11 ADD .020KEV/CH 100.SECS
BUG= 3.660KEV,26140.
000
100.
20.460

E


ADD .020KEV/CH 100.SECS
BUG= 3.680KEV,26257.
000

F

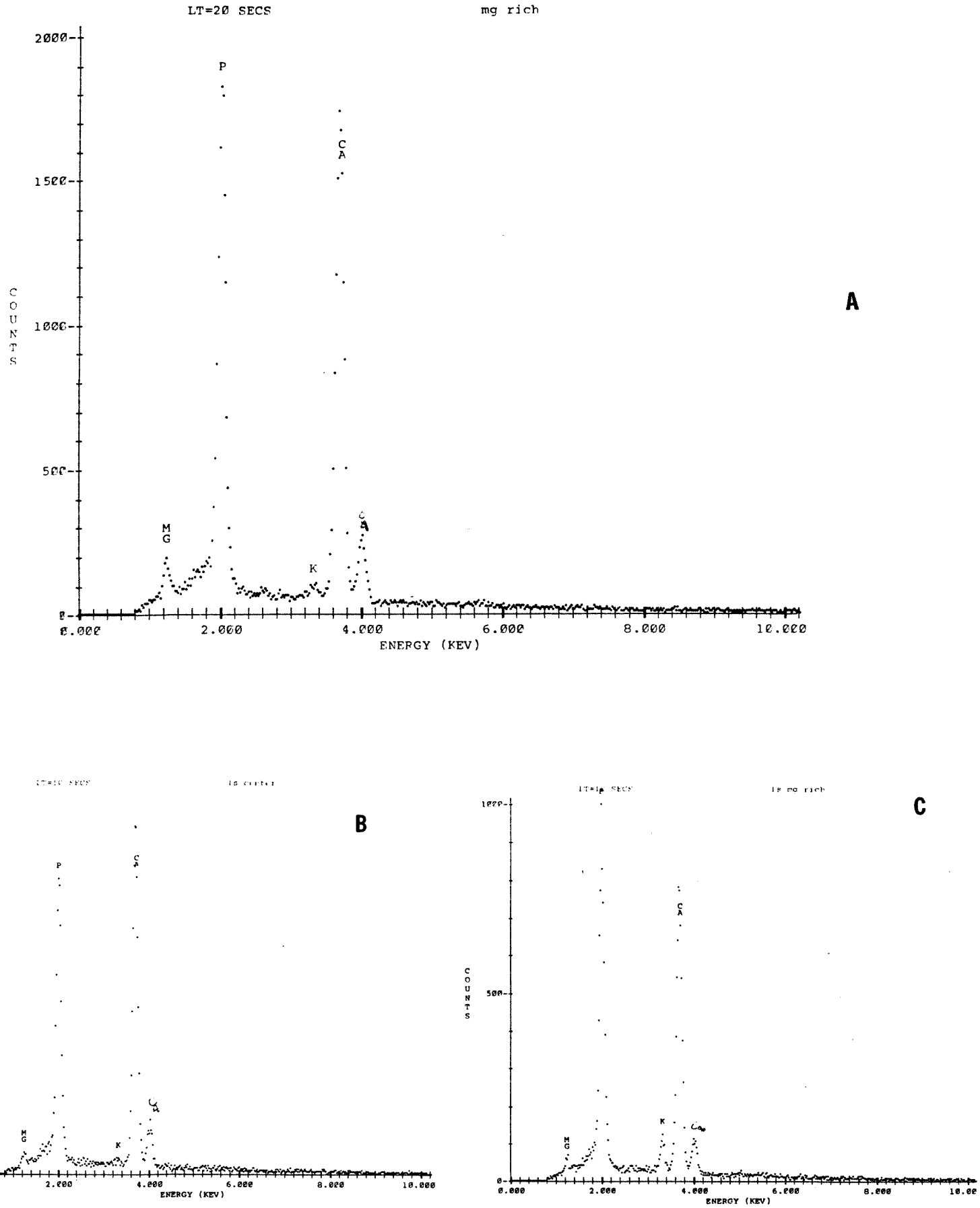
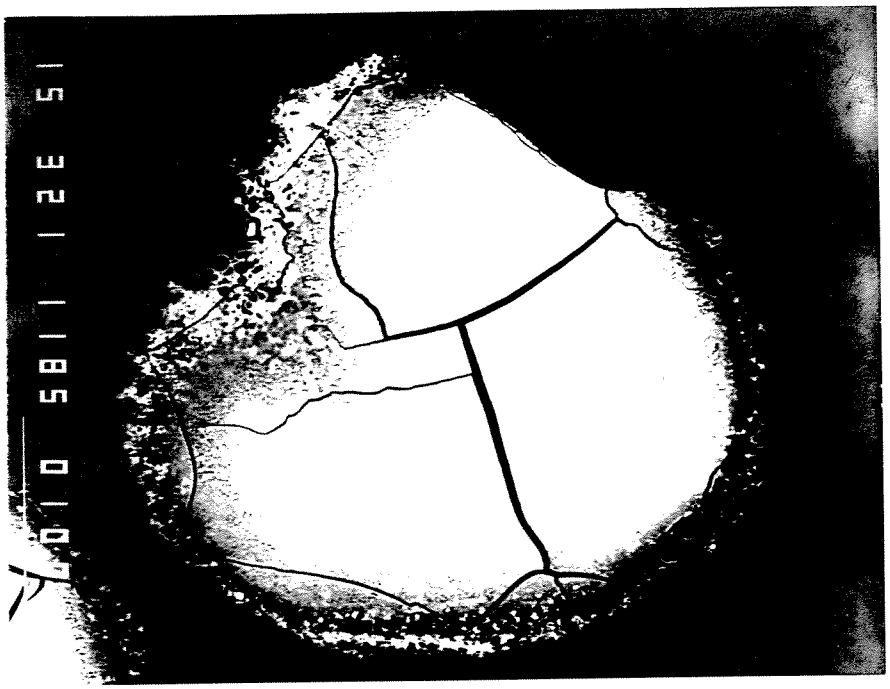
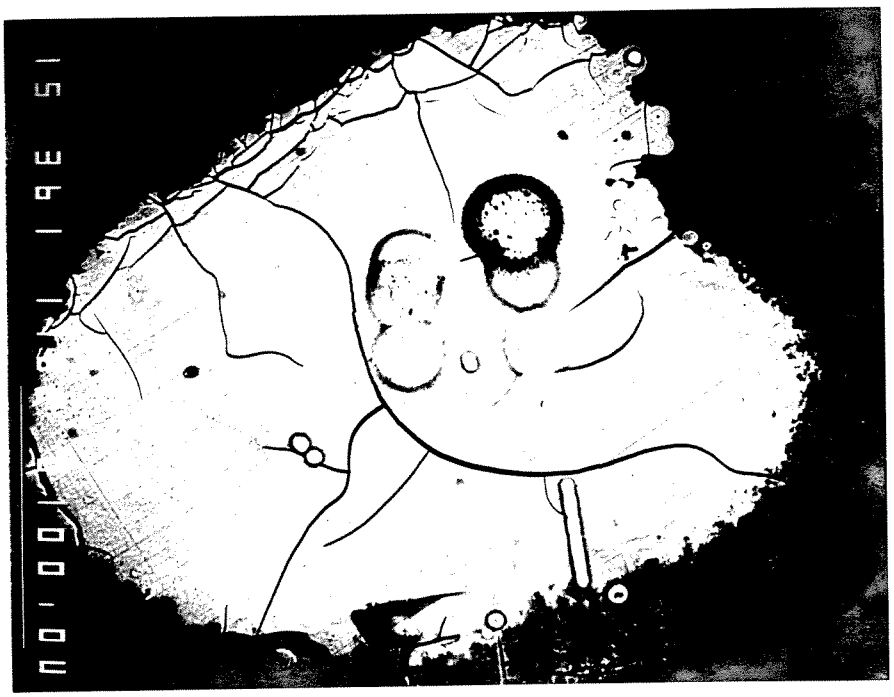

LT=20 SECS
mg rich
2000
1500
1000
500
0
COUNTS
P
C
A
M
G
K
C
A
A
0.000
2.000
4.000
6.000
8.000
10.000
ENERGY (KEV)
B
P
C
A
M
G
K
C
A
0.000
2.000
4.000
6.000
8.000
10.000
ENERGY (KEV)
C
C
A
M
G
K
C
A
0.000
2.000
4.000
6.000
8.000
10.00
ENERGY (KEV)

FIGURE 10



A



B

STM

FIGURE 11

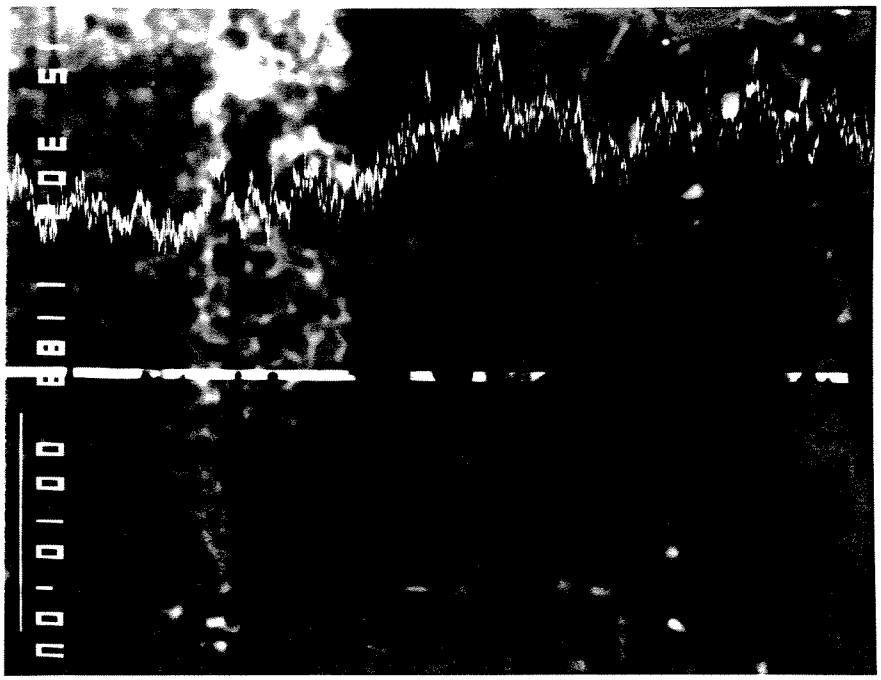
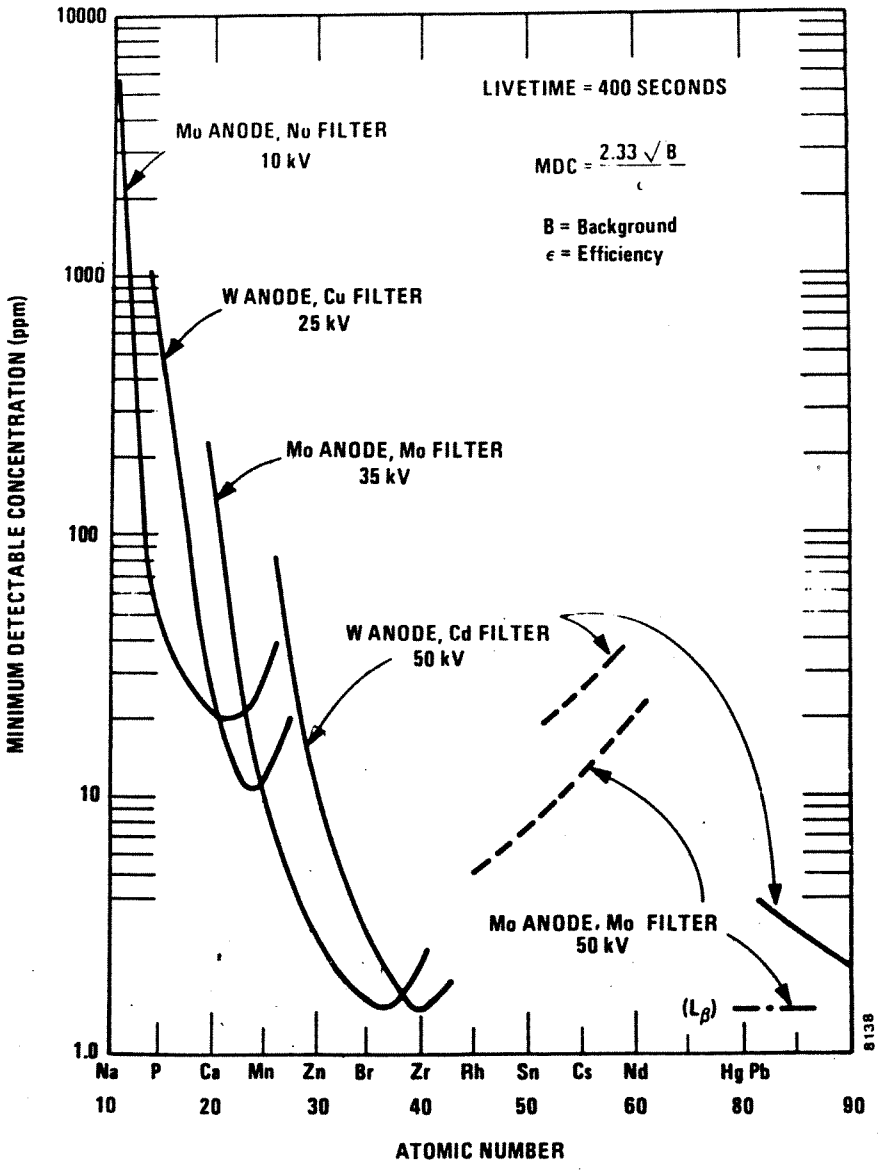


FIGURE 12



Minimum Detectable Concentration (MDC) in Geological Material for XRF

FIGURE 13

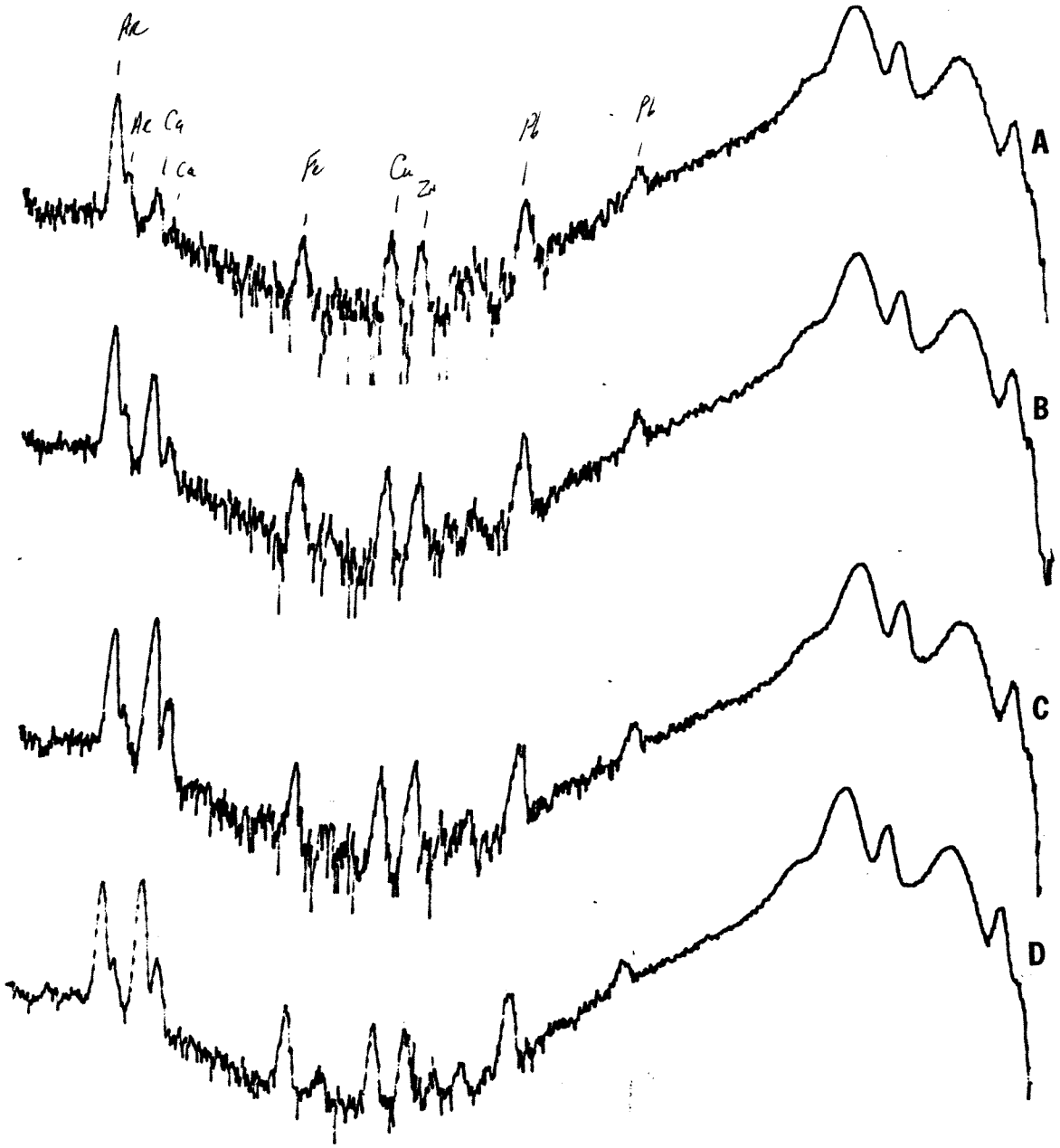


Figure 14

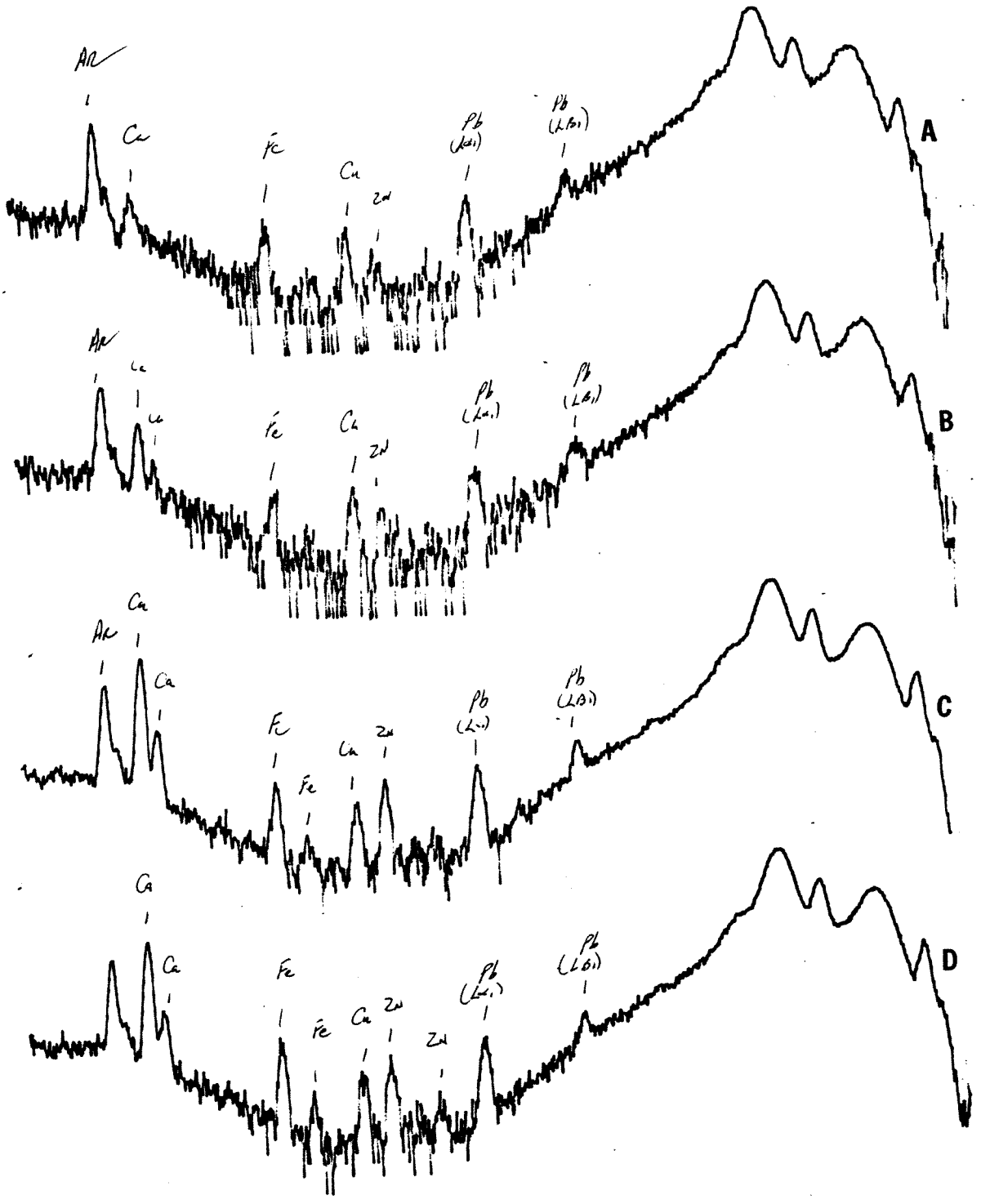


FIGURE 15

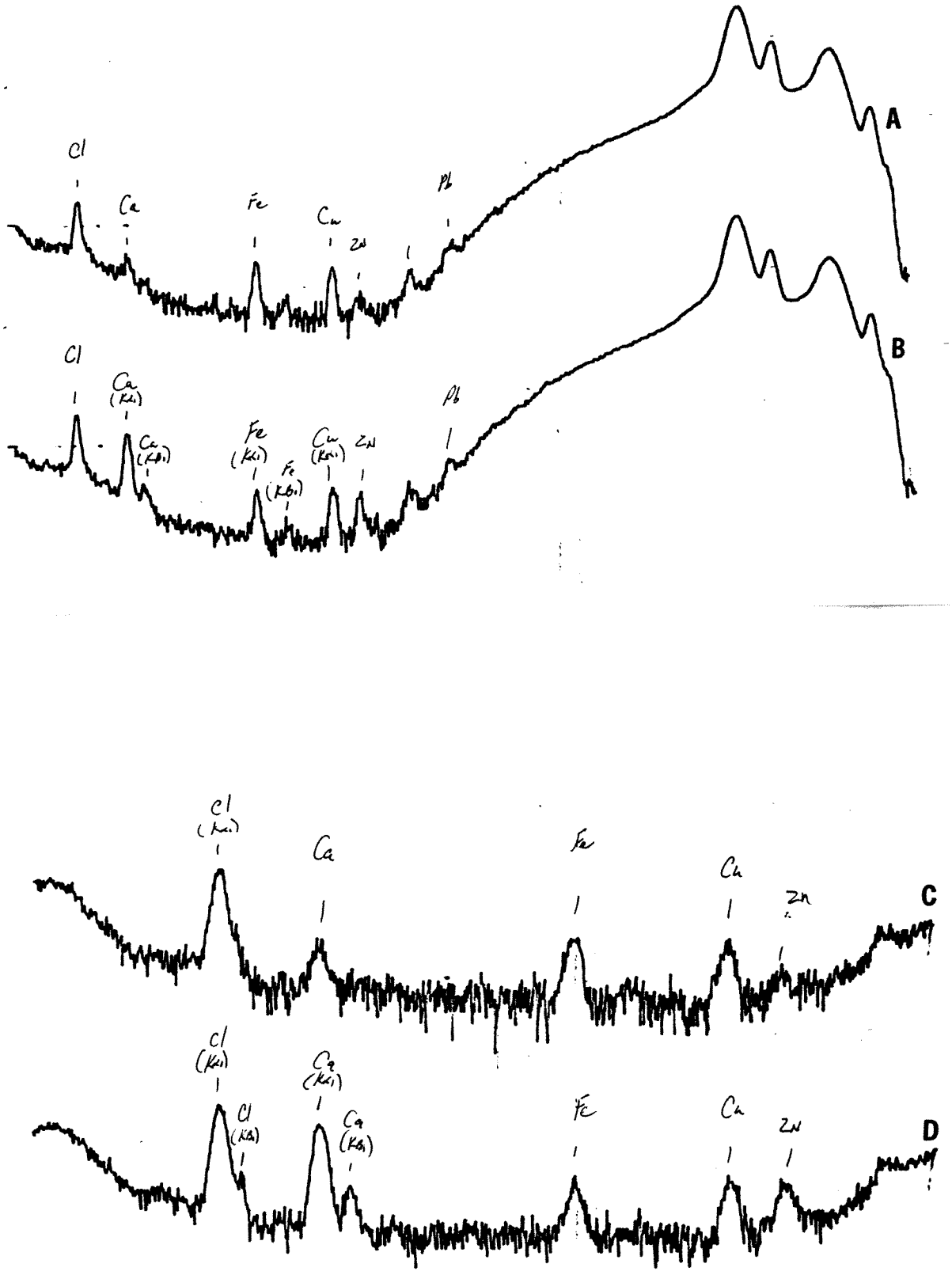
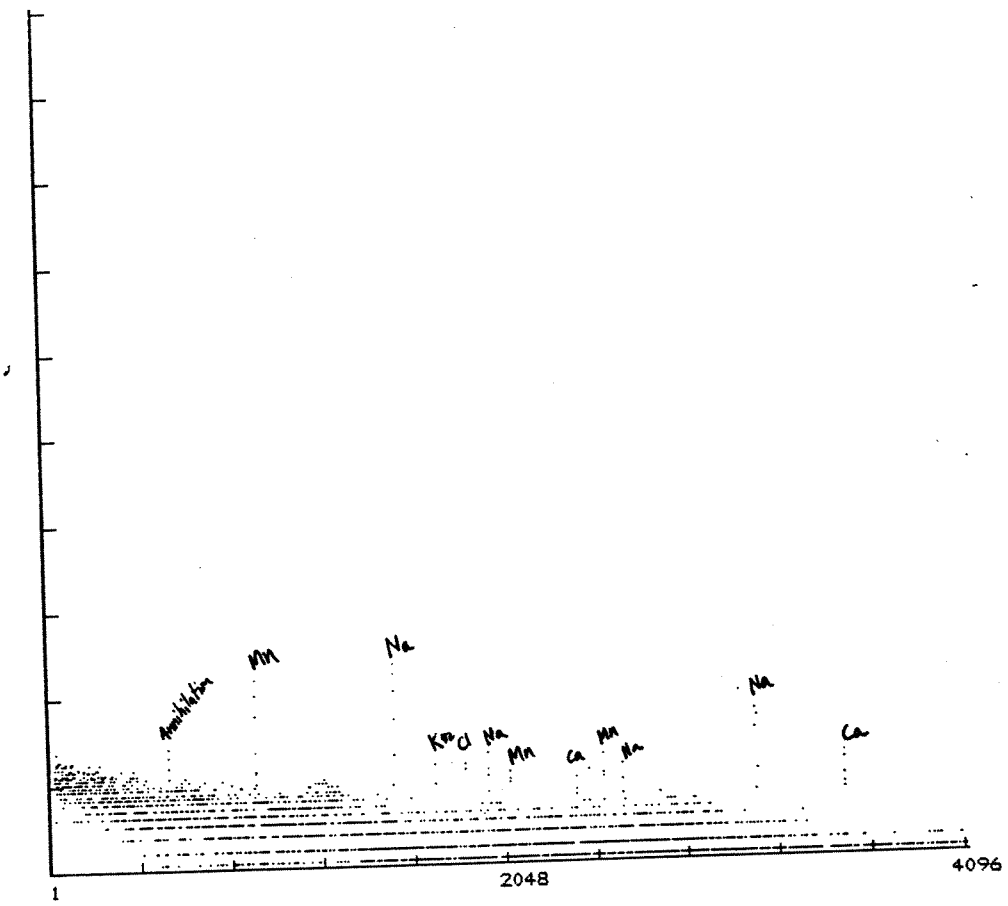

Cl
Ca
Fe
Cu
Zn
Pb
A
Cl
Ca (Kα1)
Ca (Kβ1)
Fe (Kα1)
Fe (Kβ1)
Cu (Kα1)
Zn
Pb
B
Cl (Kα1)
Ca
Fe
Cu
Zn
C
Cl (Kα1)
Cl (Kβ1)
Ca (Kα1)
Ca (Kβ1)
Fe
Cu
Zn
D

FIGURE 16

#7 EX 2MMIN 1/29 400KW DT2 29-JAN-87 10:19:42



GRAPH OF:
COUNTS VS. KEV COUNTS FULL SCALE = LOG

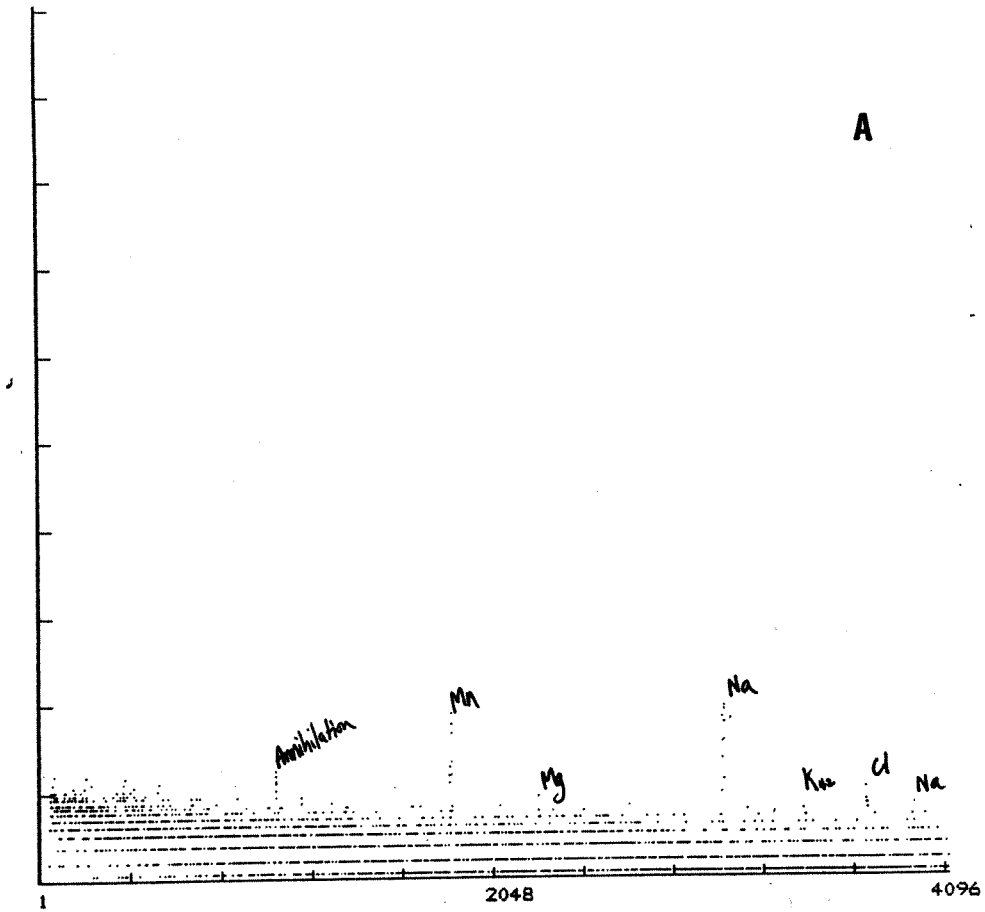
ENERGIES:
LEFT ENERGY 60.323 KEV RIGHT ENERGY 3543.250 KEV

ELAPSED:
LIVE TIME 00:10:17 REAL TIME 00:10:17 TOTAL 0

CALIBRATION:
QUAD (*10**8) 0.000 SLOPE 0.850531 ZERO 59.472

FIGURE 17

#7 EX 2MMIN 1/29 400KW DT2 29-JAN-87 09:59:58



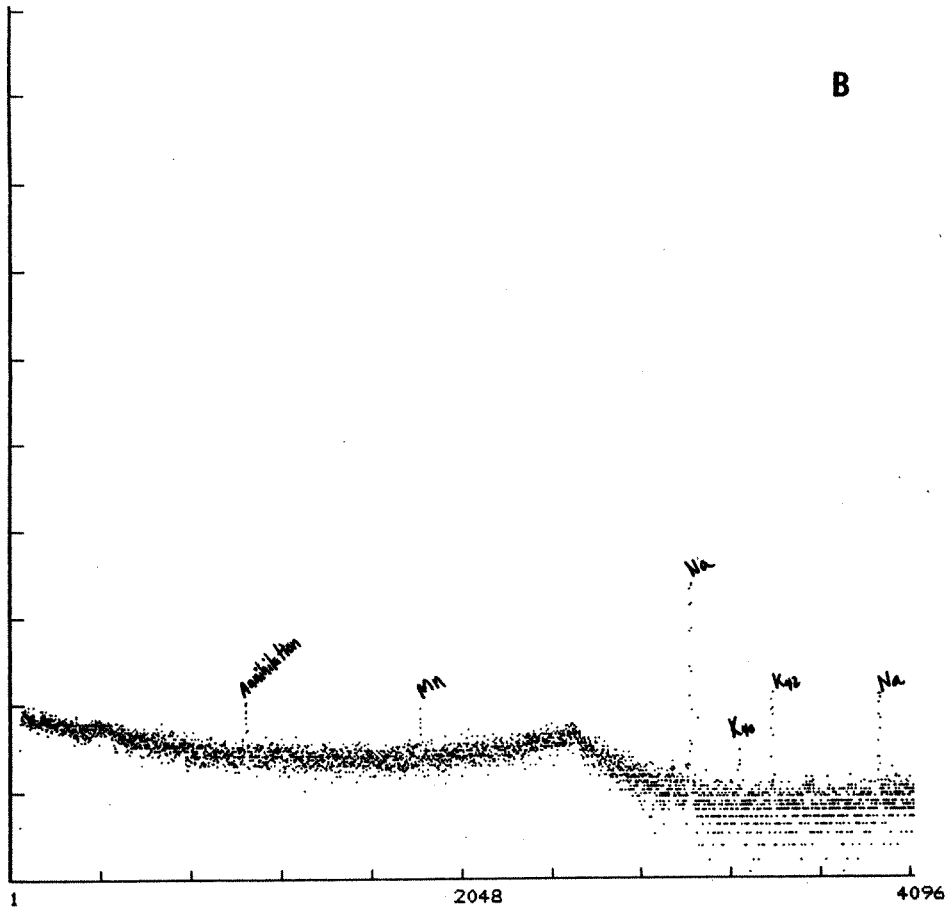
GRAPH OF:
COUNTS VS. KEV COUNTS FULL SCALE = LOG

ENERGIES:
LEFT ENERGY 60.789 KEV RIGHT ENERGY 1799.531 KEV

ELAPSED:
LIVE TIME 00:09:51 REAL TIME 00:09:52 TOTAL 0

CALIBRATION:
QUAD (*10**8) 0.000 SLOPE 0.424601 ZERO 60.364

BROTHERS #7 CLEAN VIAL 28-JAN-87 15:23:09



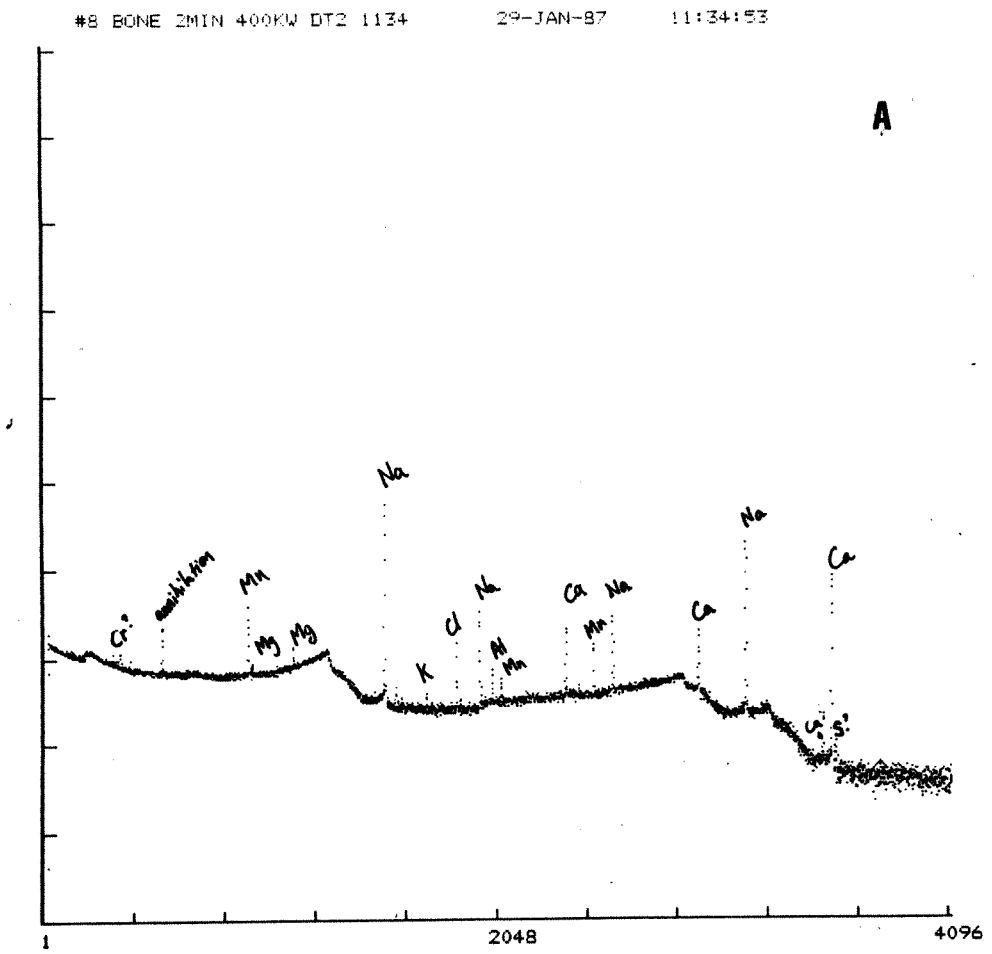
GRAPH OF:
COUNTS VS. KEV COUNTS FULL SCALE = LOG

ENERGIES:
LEFT ENERGY 60.789 KEV RIGHT ENERGY 1799.531 KEV

ELAPSED:
LIVE TIME 01:40:00 REAL TIME 01:40:10 TOTAL 0

CALIBRATION:
QUAD (*10**8) 0.000 SLOPE 0.424601 ZERO 60.364

FIGURE 18


#8 BONE 2MIN 400KW DT2 1134 29-JAN-87 11:34:53
A
Cr?
annihilation
Mn
Mg
Mg
K
Cl
Na
Mn
Mn
Ca
Na
Mn
Ca
Na
Ca
Cr
S
1
2048
4096
GRAPH OF:
COUNTS VS. KEV COUNTS FULL SCALE = LOG
ENERGIES:
LEFT ENERGY 60.323 KEV RIGHT ENERGY 3543.250 KEV
ELAPSED:
LIVE TIME 00:15:27 REAL TIME 00:16:49 TOTAL 0
CALIBRATION:
QUAD (*10**8) 0.000 SLOPE 0.850531 ZERO 59.472

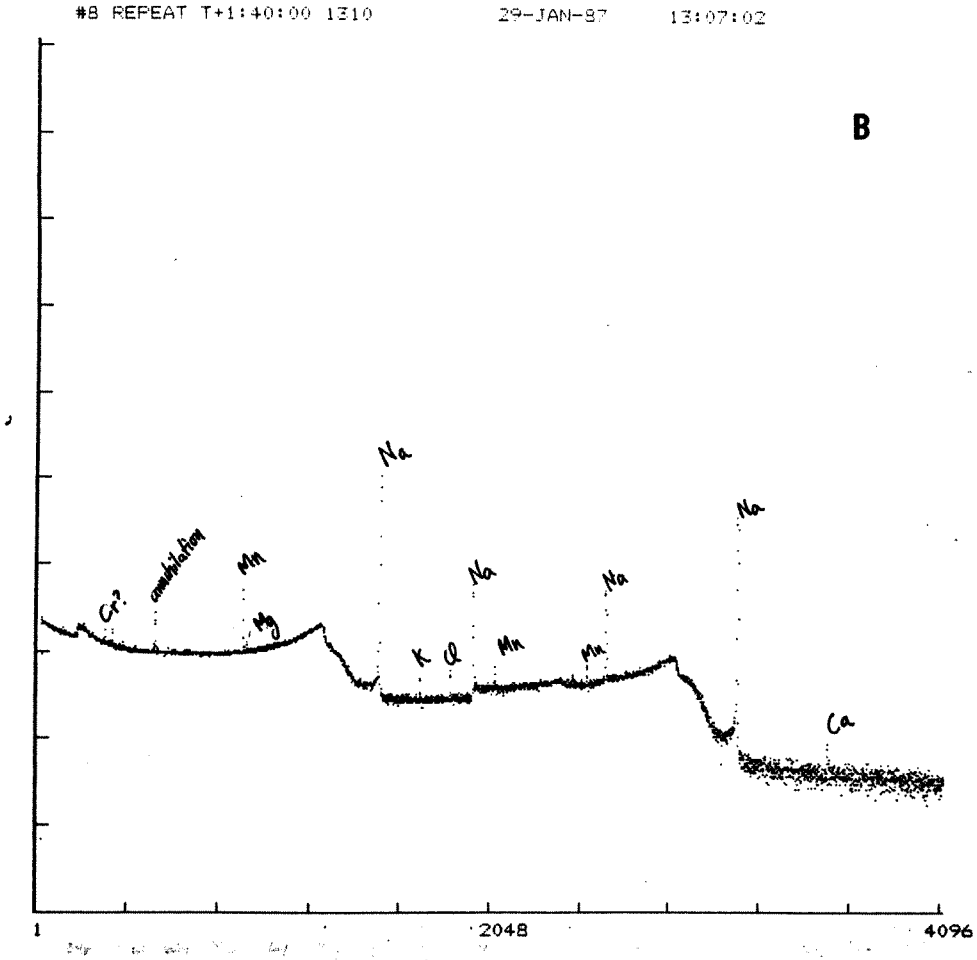
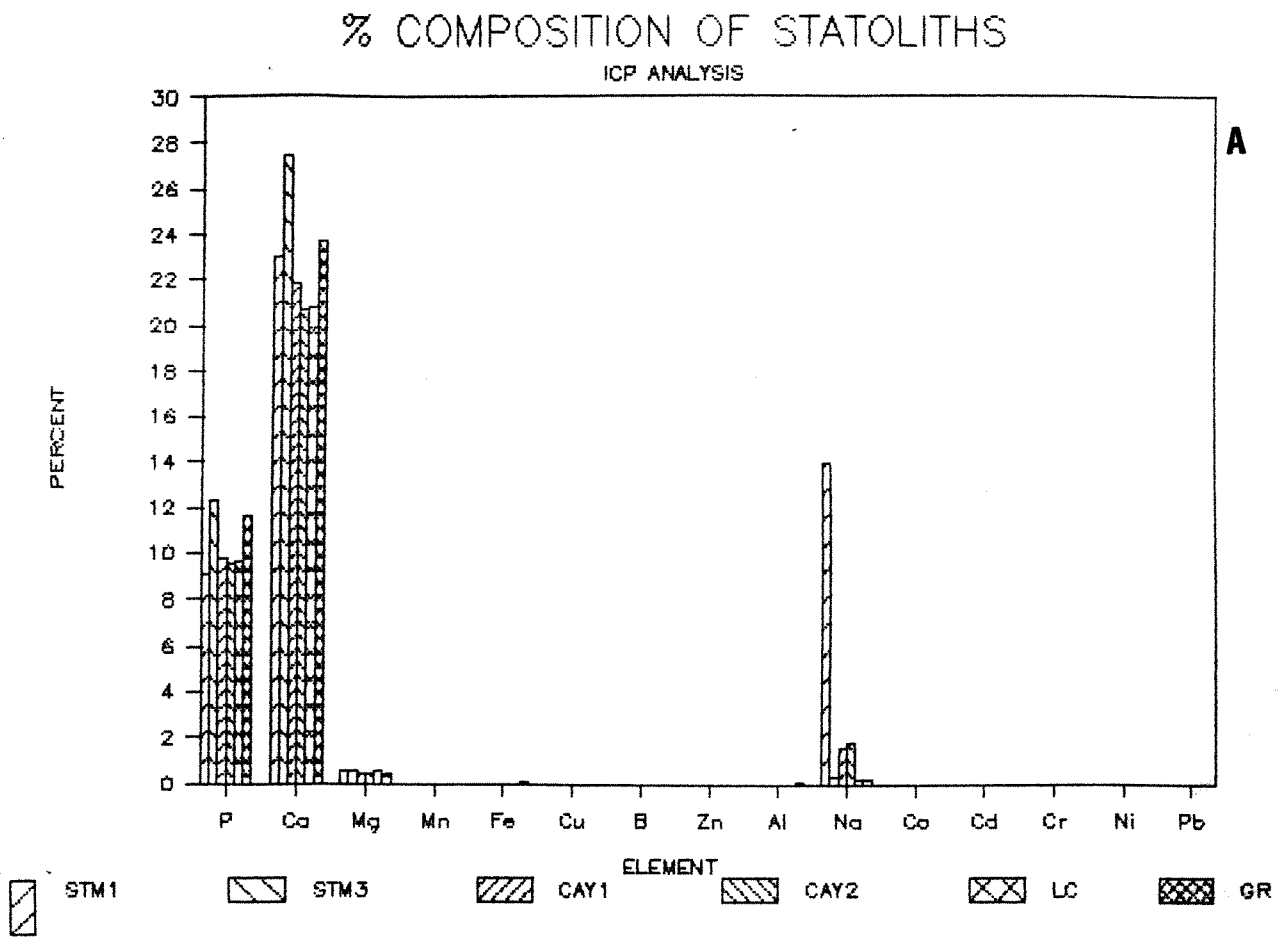

#8 REPEAT T+1:40:00 1310 29-JAN-87 13:07:02
B
Cr?
annihilation
Mn
Mg
K
Cl
Na
Mn
Mn
Na
Na
Ca
1
2048
4096
GRAPH OF:
COUNTS VS. KEV COUNTS FULL SCALE = LOG
ENERGIES:
LEFT ENERGY 60.323 KEV RIGHT ENERGY 3543.250 KEV
ELAPSED:
LIVE TIME 00:44:57 REAL TIME 00:46:34 TOTAL 0
CALIBRATION:
QUAD (*10**8) 0.000 SLOPE 0.850531 ZERO 59.472

FIGURE 19


% COMPOSITION OF STATOLITHS
ICP ANALYSIS
A
PERCENT
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
P
Ca
Mg
Mn
Fe
Cu
B
Zn
Al
Na
Co
Cd
Cr
Ni
Pb
ELEMENT
STM1
STM3
CAY1
CAY2
LC
GR

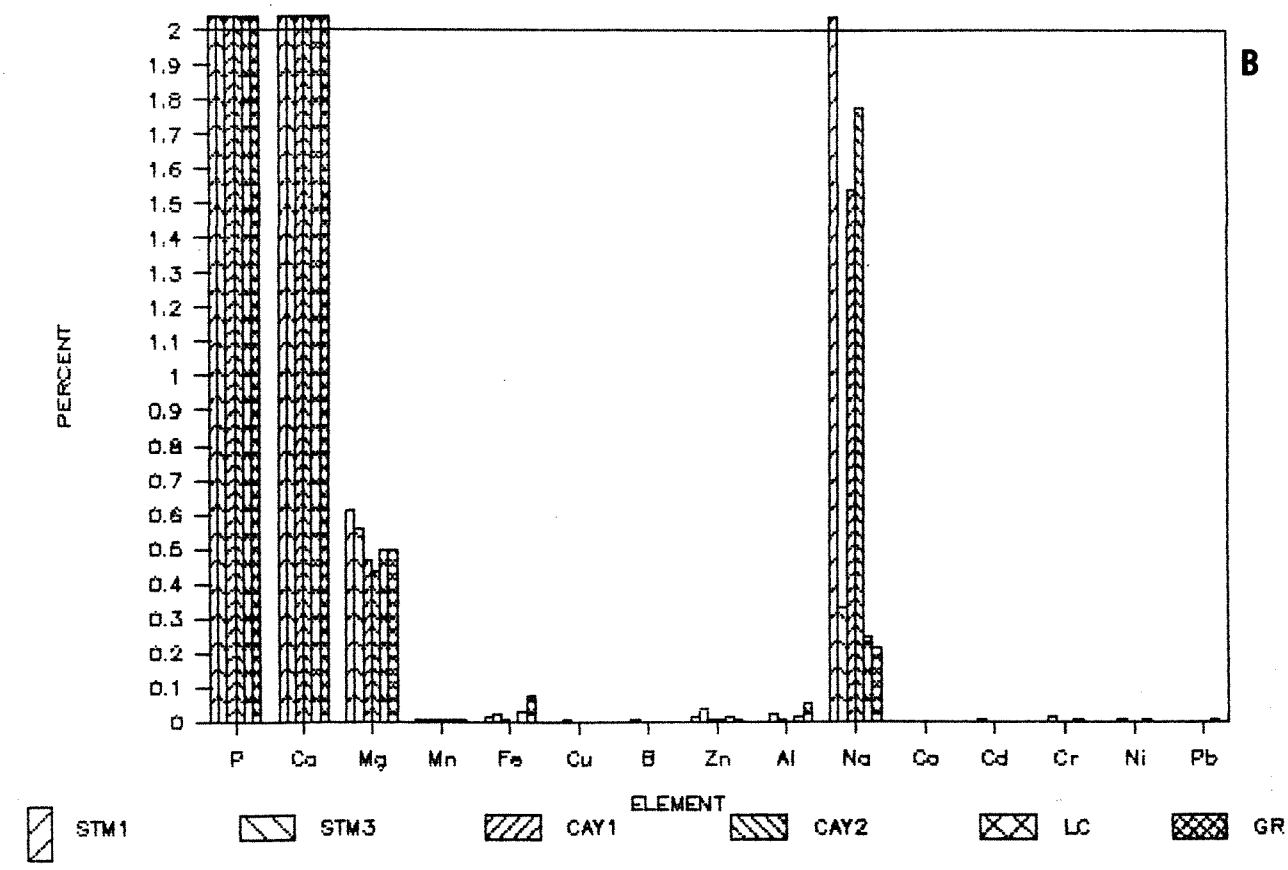
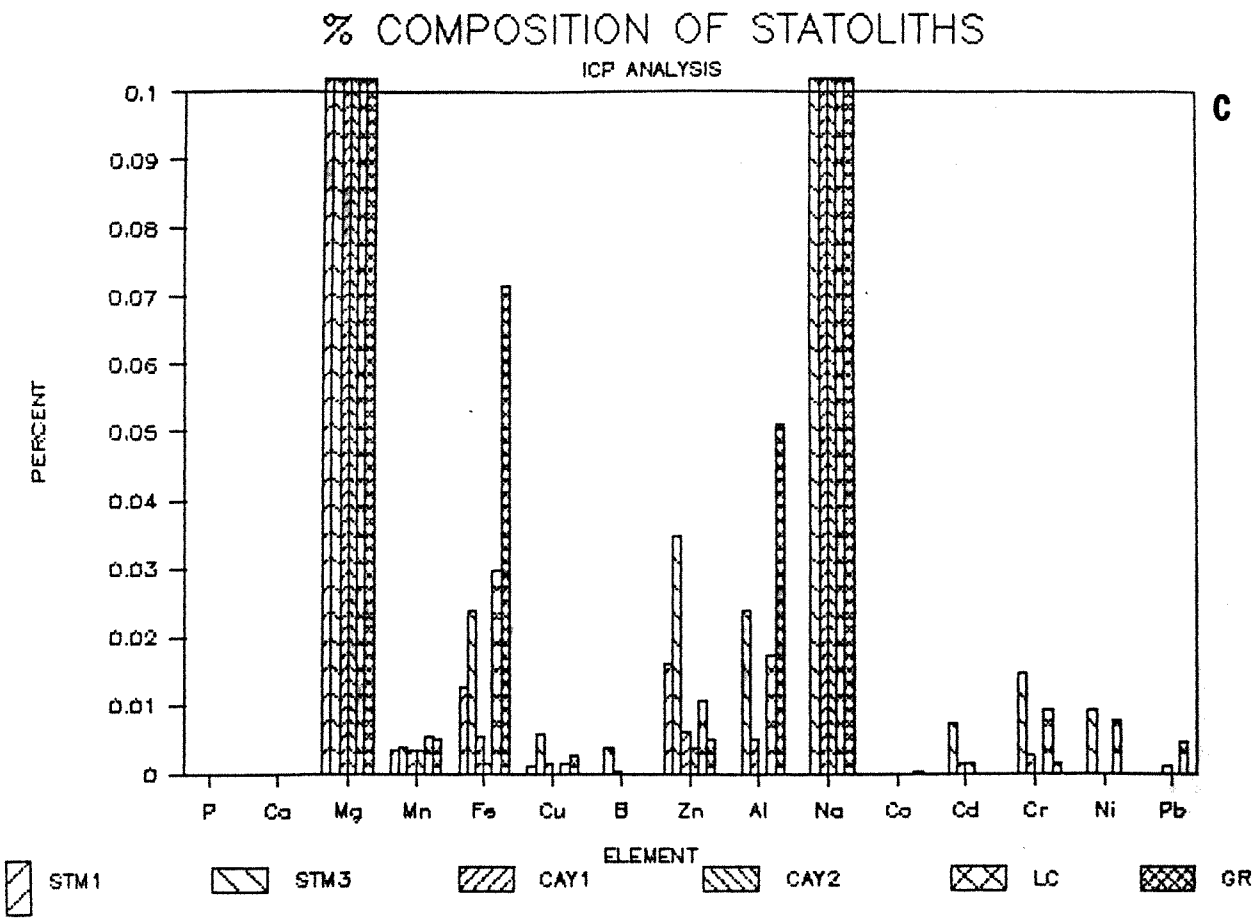

B
PERCENT
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
P
Ca
Mg
Mn
Fe
Cu
B
Zn
Al
Na
Co
Cd
Cr
Ni
Pb
ELEMENT
STM1
STM3
CAY1
CAY2
LC
GR

FIGURE 20


% COMPOSITION OF STATOLITHS
ICP ANALYSIS
C
PERCENT
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
P
Ca
Mg
Mn
Fe
Cu
B
Zn
Al
Na
Co
Cd
Cr
Ni
Pb
ELEMENT
STM1
STM3
CAY1
CAY2
LC
GR

DETECTION LIMITS
Ng ml-1 (ppb)

(3σ , 10 Second Integration)

I A	IIA	III B	IV B	V B	VI B	VII B	VIII			I B	II B	III A	IV A	V A	VI A	VII A	O
H																H	He
0.06 Li	0.1 Be											0.08 B	50 C			30 F	Ne
0.06 Na	0.10 Mg											0.1 Al	10 Si	2 * P	1 S	1 Cl	Ar
1 * K	5 Ca	0.08 Sc	0.06 Ti	0.03 V	0.02 Cr	0.04 Mn	0.2 Fe	0.01 Co	0.03 Ni	0.03 Cu	0.08 Zn	0.08 Ga	0.08 Ge	0.4 As	1 Se	100 Br	Kr
0.02 Rb	0.02 Sr	0.01 Y	0.03 Zr	0.02 Nb	0.08 Mo	Tc	0.05 Ru	0.02 Rh	0.06 Pd	0.04 Ag	0.07 Cd	0.01 In	0.03 Sn	0.02 Sb	0.04 Te	0.01 I	Xe
0.02 Cs	0.02 Ba	0.01 La	0.03 Hf	0.02 Ta	0.06 W	0.06 Re	0.01 Os	0.06 Ir	0.08 Pt	0.08 Au	0.08 Hg	0.05 Tl	0.02 Pb	0.06 Bi	Po	At	Rn
Fr	Ra	Ac															

0.01 Ce	0.01 Pr	0.01 Nd	Pm	0.04 Sm	0.02 Eu	0.04 Gd	0.01 Tb	0.04 Dy	0.01 Ho	0.02 Er	0.01 Tm	0.03 Yb	0.01 Lu
0.02 Th	Pa	0.02 U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lw

* µg ml-1

Negative Ion Mode

elan ICP MS Elemental Analysis System

FIGURE 21

SCIEX