

**Study of Trace and Minor Elements in
ALS (Amyotrophic Lateral Sclerosis) Patients**

by

Rena J. Lee
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for the Degree of

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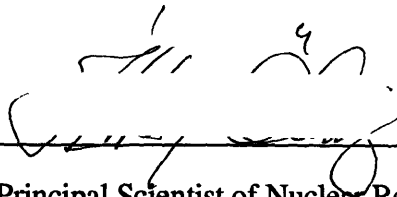
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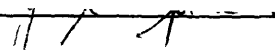
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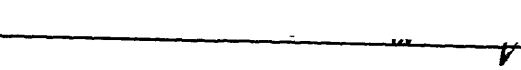
Ilhan Olmez
Principal Scientist of Nuclear Reactor Laboratory and Nuclear Engineering
Thesis Supervisor

Certified by



Jacquelyn Yanch
Professor of Nuclear Engineering
Thesis Reader

Accepted by



Allan Henry, Chairman
Departmental Committee on Graduate Students

science
MASSACHUSETTS INSTITUTE
OF TECHNOLOGY

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Abstract

The importance of trace and minor elements in human disease has been extensively studied. Excessive or deficient amounts of certain elements in the human body have been known to be a causative factor for disease. Despite their importance, the levels of trace and minor elements in the human brain yet to be well established. This study has been performed to provide a library of elemental concentration in the human brain and spinal cord. In addition, degenerated samples obtained from patients who died from ALS (Amyotrophic Lateral Sclerosis) were analyzed to find any elements that might be responsible for the disease. Elemental profile of the ALS and the control samples taken from the motor cortex, cerebellum, and spinal cord were obtained by instrumental neutron activation analysis (INAA). Irradiations have been performed at a thermal neutron flux of 8×10^{12} n/cm² sec at the MIT-II reactor. Concentrations of eighteen elements have been determined from short and long experiments.

Summary statistics were applied to data sets to examine the statistical properties of the obtained data sets. Average, standard deviation, range, and standardized Kurtosis coefficient were calculated. Average values for each element were compared between ALS and control samples. Aluminum and Sb showed wide distributions in the motor cortex of control samples. Bromine, Cr, Cs and Eu showed wide distributions in the motor cortex of ALS samples. High levels of Mg, Al, V, Sb, Cr, Eu, Fe, and Se and low levels of Ti, Br, Cd, and Rb were observed in ALS samples taken from the motor cortex. Different levels of elements between ALS and control samples were also found in cerebellum and spinal cords. The difference in elemental concentrations deposited in the human brain between ALS and control samples may provide a clue for the etiology of ALS. More studies, however, need to be done for justification.

A correlation analysis has been conducted to determine correlation among the observed elements. Results of this analysis showed that there were different elemental correlations in brain tissues of ALS and the control sample.

The principal component factor analysis has been applied to the elemental data matrix in order to examine the applicability to biological data sets. Results reveal that by plotting factor scores one can differentiate samples that have the same origin. And the elements in that factor can be used as an indication of that origin.

Thesis Supervisor: İlhan Olmez
Principal Scientist of Nuclear Reactor Laboratory and Department of
Nuclear Engineering

Thesis Reader: Jacquelyn Yanch
Associate Professor of Nuclear Engineering

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Mam, Dad, grandmother,
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Chapter 1 Introduction

1.1 Brief Background of Present Study

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease which shows dementia, muscular weakness, and atrophy. While not as common as Alzheimer's disease, it is similar in the sense that both usually occur in the elderly. The formation of neurofibrillary tangles (NFT) throughout a variety of sites in the central nervous system and spinal cord are the characteristic factors of ALS that separate this disease from other neurodegenerative diseases. The progress of this disease is slow, but it leads to death within a few years, once it is symptomatic. Patten (1986) claimed that ALS is a syndrome rather than a disease because ALS is characterized by the failure of motor neurons in various parts of central nervous system, though it is still commonly referred to as a disease.

There have been high death rates for Chamorro residents of Guam and Rota from ALS since the World War II. This has attracted much attention to Guam, and intensive longitudinal studies of the indigenous population of Marianas islands of Guam and Rota have been performed to find possible causation of the disease for over 40 years. Despite long studies on the etiology of ALS, the cause is still unknown.

Among possible causative factors such as genetic, nutritional, and environmental, an effect of metals on motor neuron disease has been repeatedly studied. Analytical studies on environmental factors and metal analysis in central nervous system (CNS) tissues from

ALS disease indicated that chronic exposure to metals such as aluminum and manganese, together with a deficiency of minerals such as calcium and magnesium, may play a major role in the neurodegeneration seen in ALS (Yanagihara, 1982). Due to limited number of biological samples (less than 10 samples in most of cases) and difficulty in analyzing sample with small size, no single element has been selected as an major causative factor.

The methods in element analysis of biological samples have improved during past 30 years. Recent advances in methodological approaches for studying trace elements in biological samples showed the possibilities of instrumental neutron activation analysis (INAA) as a means in determining the concentrations of elements. The advantages and disadvantages of this technique will be discussed in the next chapter. One of the most distinct advantages of INAA over many other analytical techniques is that it allows simultaneous determination of numerous trace metals and minor elements in a biological matrix within a small sample. INAA is capable of measuring elemental concentrations within the range of 10^{-6} to 10^{-11} g making it useful for analyzing biological samples (Olmez, 1989).

In order to investigate the underlying processes in diseases or degenerated samples in which trace elements are involved, it is important to identify the element or elements in the diseased sites and the particular biochemical processes in which they are involved. However, our present knowledge lacks the basic relationships between trace element levels in tissue and specific biochemical roles they may play in diseased sites. There have been major problems in the study of the elucidation of any disorder in terms of fundamental alteration in trace element levels in disease tissues. The first problem is the great variability of biological systems: e. g., race, geographical location, age, sex, cultural habits, regional distribution, etc. Therefore, the normal concentration ranges of most elements in nervous tissue are not yet known. The concentrations of Na, Mg, P, Cl, and K in cerebrospinal fluid and those of Mg, K, Mn, Fe, Cu, Sr, and Cs in the brain have been defined to some

extent (Iyengar and Gavindaraja, 1978), but the levels of the majority of elements in the brain or CNS have not been reliably confirmed. Therefore, the study of elements in determining the concentration range in normal nervous tissue with large number of samples is essential to understand the role of metals in human disease.

Factor analysis is a statistical method that attempts to simplify the description of a data set by determining a minimum set of basis vectors that span the data space to be interpreted. The determined factors may represent a group of elements that are varying together from one sample to another, and more importantly, indicate the differences of biochemical processes between the control and diseased populations. The obtained factor loading coefficients for each variable, on hypothetical common factors, reveal how seemingly unrelated variables relate to one another. Factor analysis is usually used in environmental studies to investigate the possible sources and transport of atmospheric pollutants into and out of a site. However, this method can also be used to determine the common factors that influence trace and minor element concentration in biological systems. The differences in these extracted common factors in biological data matrix may represent factors due to the changes caused by the disease.

1.2 Objectives of Present Research

The primary goal of this study is to provide the normal ranges of elements in the human brain and confirm the existing results mentioned previously, that high concentrations of some elements are associated with pathogenesis of ALS. The ultimate objective of this work is to understand the underlying biochemical processes among the elements that may be responsible for the neurotoxicity in ALS disease. The hypothesis under scrutiny is that certain trace elements may play a neurotoxic role in ALS disease and may also be related to other disorders of the human nervous system.

In order to achieve these goals, the following objectives were defined:

1. Obtain as many samples as possible from ALS diseased and healthy subjects.
2. Improve the INAA technique to determine as many trace and minor elements as possible with great accuracy within the biological samples.
3. Determine and compare the concentration of trace and minor elements in ALS and control samples and examine the distributions of the elements.
4. Provide a data set that can be termed as a baseline for a given control population.
5. Apply a statistical analysis to see underlying relationships between elements.
6. Study the obtained factors to determine whether or not there are differences in elemental patterns between the control and diseased groups.
7. Connect the findings to the cause of ALS and compare with other studies.

Chapter 2 Description of ALS

2.1 Types and Prevalence

ALS is a neurodegenerative disease, in which the nerves that control muscular activity degenerate within the brain and spinal cord. The degeneration in those sites results in weakness and atrophy of the muscles. There are three forms of ALS: sporadic or classic form, genetic or inherited form, and Guamanian (Kurland and Molgaard 1982). The pathology of these types will be explained in the next section.

The diagnosis of ALS is obvious from routine examination and inspection of upper and lower motor neurons since motor neurons are usually the problem. Symptoms generally start with muscular atrophy alone. Other signs such as sensory loss, and excessive weight loss, appear as the disease progresses. Once symptomatic, it leads to death in two to three years. Since very little is known about the causation, the treatment is also unknown.

The incidence and mortality rate of ALS throughout the world is approximately 1 per 100,000 with the exception of very high incidence rates in the western Pacific and east Asia. There were two major epidemiological studies on the incidence rate of ALS in the US. A study of the Rochester, Minnesota population covering 53 years indicated that the mean annual incidence rate for ALS was 1.9 per 100,000 person-years (Juergens et al. 1980). According to another study (Brody et al. 1986), the incidence rate for ALS in the United States and Canada was approximately 1.5 per 100,000 per year.

Jokelainen (1986) have studied death rates in Finland from 1963 to 1982. He found an increasing rate from 2 to 6 per 100,000 from 1965 to 1982, respectively.

The high incidence of ALS among the Chamorro people of Guam was observed by several observers and attracted many peoples attention in 1950. Kurland and Mulder (1954) showed that the ALS prevalence ratios and death rates for Chamorro residents of Guam or Rota were 50 to 100 times (200 per 100,000 population) the estimates for the continental United States and other developed countries.

Based on the Guam data, Brody et al. (1986) calculated the incidence in terms of deaths per year because this was more accurate than counting onset . They found that the incidence per 100,000 on Guam was 40 for the years of 1950 to 1964. They also studied the effects of age and sex on the incidence rate. The highest death rate occurred in the 55-59 and 60-64 year age groups among male and female populations, respectively. The ratio of males to females varied each year. The overall rate among males, however, was found to be 12 times that for females. After a high incidence on Guam, Garruto et al. (1986) have observed a decline over the past thirty years.

2.2 Pathology of ALS

The definite boundary for the pathology of ALS has first developed by Charcot in 1850 (Hughes, 1981). After the first study, it has been thoroughly reviewed many times. Studies of the pathology of ALS revealed that the involvement of various areas were quite specific in contrast to other diseases.

The classic form shows a selective involvement of the motor neuron system. In order words, there are selective degeneration of spinal motor neurons and of motor neurons in the brain stem. Upper and lower motor neurons are also involved and these areas show extensive neuronal loss.

Another type of ALS is Guammanian form that shows familial aggregation as opposed to the generally sporadic occurrence of classic ALS in most other parts of the world. A pathologic alternation was found in Guammanian ALS that was different from the classic form (Malamud et al., 1961). The difference between classic and Guammanian form is the formation of neurofibrillary degeneration (NFD) which is seen in Alzheimer's disease. Neurofibrillary degeneration in Guammanian ALS is included in a variety of sites throughout the central nervous system. The number of neurons containing NFD varies from case to case. However, the areas containing the most NFD generally form a second group of sites that are different from those involved in classic ALS.

A genetic hypothesis developed came out due to the familial aggregation of ALS noted on Guam and the failure to identify any infectious basis for the disease. The pathologic studies on familial Guammanian ALS have shown another variant form the classic form of ALS which is the genetic form. In most familial cases, all of the classic pathologic features of ALS are present. In addition to that of classic features, familial forms show a certain percentage of rather mild involvement of upper motor neurons in familial ALS (Anderson, 1979). Also, the alternation of the anterior horn cells has been observed which is different from other types of ALS.

2.3 Etiology of ALS

The causative mechanisms or causative factors of ALS are not well known since there is little solid evidence for any single etiologic factor. One of the first thoughts that attracts most investigations was Guammanian type. There have been intensive etiologic studies to find a genetic causative factor (Kurland et al., 1982). Certainly, this is still a good possibility. However, the crucial pathological studies of non-Chamorro residents on Guam who suffer from ALS have not yet been performed. Due to the limited studies of

this nature; time limitation, difficulties in examining affected sites, and so on, the genetic effects of this disease have not been clearly explained.

Aside from a genetic etiology, nutrition studies have also been performed. A seed called Cycad consumed by Charorrors as a food has been suspected as a causative factor. Spencer et al. (1987) have proved that the seeds contains a neurotoxic element. However, real toxic effects on the people who used it to prepare their food are not clear since the toxic component causing a neurodegeneration was thoroughly washed off before use.

More recently, Gadjusek et al.(1981) noted that soil and water in the area having a high incidence rate of ALS were low in Ca and Mg and high in Al, Mn, and Fe in the same villages. Based on this observation, a suggestion was made that pathogenesis may involves chronic nutritional insult, or early exposure to an environment that leads to disordered disease. In fact, the high incidence rates of ALS among people in geographically isolated island such as Guam can be explained by environmental or acquired factors. There are four separate aspects of epidemiological analysis that support the idea that toxic or deficiency factors in the etiology of ALS on Guam. The four factors that support the idea of environmental effect are: the steady decline in the average annual incidence of ALS on Guam, the upward shift in the average age at onset in recent years, similarity of mean exposure time for migrant ALS patients coming to or leaving Guam, and the similarity of the latency period for a migrant.

Among those possible causative factors, the etiology of ALS is still unknown and is a topic of ongoing research. In this study, the analysis of trace and minor elements will be performed to find a possible causation of ALS under the hypothesis that a deficiency or abundance of trace elements in the central nervous system could play major role on the neuronal loss that is shown in ALS.

2.4 Pathogenic Role of Metals in ALS

Excessive or deficient amounts of essential metals in their elemental or organic forms is known to cause diseases involving the nervous system. The level of metals and metabolism of essential metals and minerals are differ from person to person. Some people are born with the inability to properly metabolize certain metals. The elements that are known in literature to cause a disease are manganese, aluminum, copper, calcium, and some others.

The significant functions of toxic metals and essential minerals in human physiology are not well understood due to the complicated biochemical interactions of these elements. The influences on human health and disease of prolonged exposures to low levels of hazardous metals are also uncertain. There are still probabilities that heavy metal exposure, essential mineral deficiency, and their clarification might give a clue in understanding disease of unknown etiology.

Heavy metals in the environment have long been suspected of being involved in the pathogenesis of ALS. Although there is a vast quantity of literature regarding the effects of deficiencies or excesses of trace minerals on degeneration disease, none is an acceptable model of ALS. In the following, the recent findings regarding the concentrations of metals in central nervous system tissues from patients dying of motor neuron disease will be explained.

Chronic manganese poisoning is characterized by a severe psychiatric disorder followed by a permanently crippling neurological disorder. Chronic manganese toxicity in humans has been known to cause “manganism” affecting the central nervous system. Studies in affected humans and non human primates indicate that manganese is concentrated primarily in the basal ganglia and cerebellum (Canavan, 1934).

Yase et al. (1968) employed instrumental neutron activation analysis (INAA) to measure manganese levels in CNS tissues of an ALS patient from Kii Peninsula, Japan, where relatively high concentrations of manganese reportedly occur in the soil. Kihira et al. (1990) studied manganese levels in the spinal cord and other anatomical regions of the brain and found no difference in the spinal cord, but higher levels in the anterior horn were found. They also found a negative correlation between manganese and calcium in the spinal cord. They speculated that the neuronal loss was associated with the imbalance of manganese and calcium. In contrast, significantly elevated concentrations of manganese were also found in spinal cord tissues another. As a conclusion, the level of manganese concentration in human brain is not yet well established as a causative on the degenerative neurological disease.

The toxic effects of aluminum have been described in many studies. Crapper et al. (1973) analyzed the brains of people who died with Alzheimer's disease, and found concentrations of aluminum similar to those in the brains of experimental animals that had been injected 150-225 ug aluminum and, that as a result, had developed neurological symptoms similar to those of Alzheimer's disease in humans. Kurlander et al. (1979) also observed high levels of aluminum in an ALS case. Yumoto et al. (1993) studied the toxic effects of aluminum on the brain and liver of rats. They observed morphological changes in the brain proving the association of aluminum with neurodegenerative disease. Although elevated concentrations of aluminum have been found in the brain tissues of patients with ALS and Alzheimer disease, the pathogenic role of aluminum in these disorders has yet to be established.

The maximal amount of selenium that man can consume in the whole diet without disease has been believed to be 3 ppm . Selenium is also known as the most toxic element among essential metals. As a result, selenium has been suspected as a toxic element responsible for pathogenesis of ALS. The relationship between the geochemical

environment and geographical veterinary pathology is amplified by disease arising from deficiencies and excesses of selenium in various parts of the world. Zhuang et al. (1991) studied the levels of trace elements in the human brain and cancer tissues by INAA and observed high levels of Ca, Fe, Cu, Zn, Se, Mn, Br, and Sc and low levels of Rb, K, and P. Kilness and Hochberg (1977) have reported elevated levels of selenium in blood and urine samples of one of their ALS patients. A subsequent investigation of unitary selenium concentration in 20 ALS patients showed low levels of selenium (Norris 1978). Since the level of selenium in different anatomical sites has not been defined, the effects of selenium concentration on degenerated tissues are not clear

Analysis of drinking water and soil samples on Guam and the Kii Peninsula, where high incidence rate of ALS was shown, showed unusually low levels of Ca and Mg, and relatively high levels of Al and Fe (Garruto 1984). Other trace elements also have been studied in a relatively few cases of ALS. None of the above studies clearly explain the association between any one of the above trace elements and ALS, perhaps certain other trace elements can play important neurotoxic roles in this disease. It is a well known fact that the level of trace elements is very important factor in human health. On the basis of the observations, it is obvious that the role of trace elements in ALS deserve clear attention in the study of the etiology of ALS.

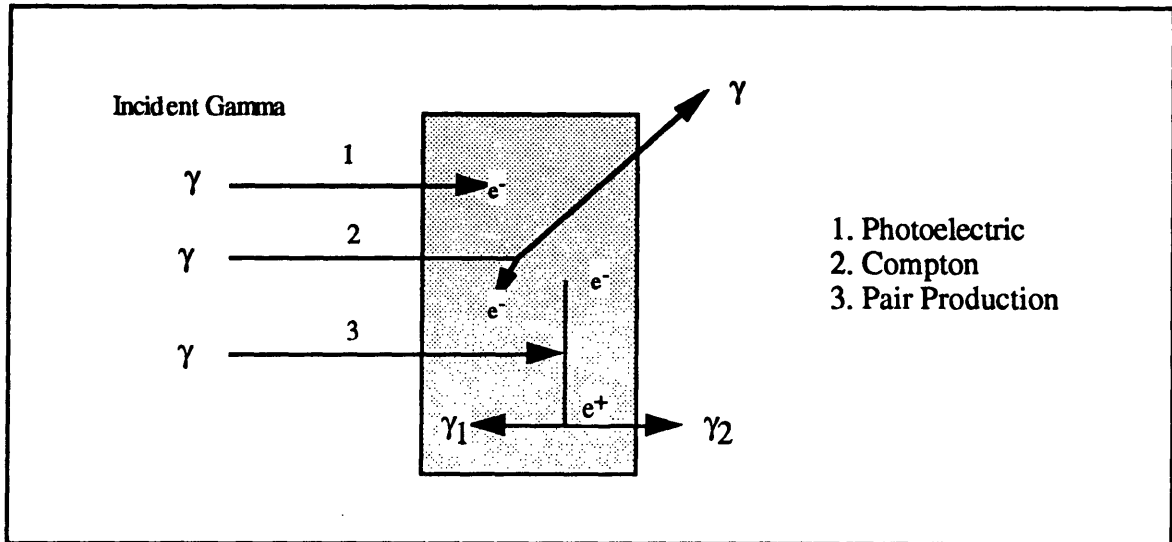
Chapter 3 Methodology (INAA)

3.1 Principles of Neutron Activation Analysis

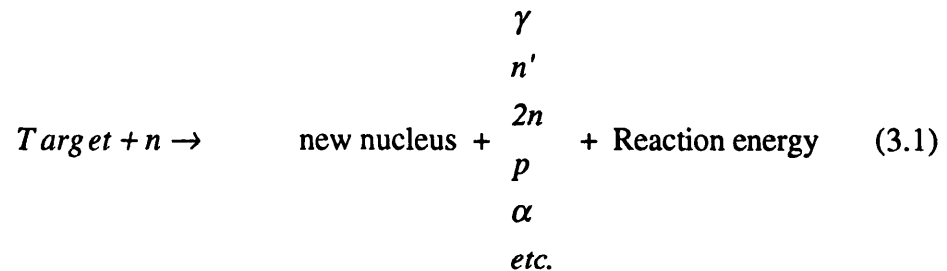
The main advantages of instrumental neutron activation analysis for process applications result from the relatively high penetrating power of neutrons and the ensuing gamma radiation. When a material is bombarded with neutrons, some of the nuclei in the material are converted to radioactive isotopes. Then the radioactive isotope will decay at a certain rate emitting gamma-rays of characteristic energies.

The emitted gamma rays mainly interact with detector in three ways: photoelectric effect, Compton scattering, and pair production (Figure 3.1). In photoelectric process, all gamma energies are deposited producing a photo-electron. Depending on the scattering angle, gamma rays lose their energy by giving part of their energy to an electron in the Compton scattering process. If the energies of gamma rays are higher than 1.02 MeV, pair production can occur. In pair production, gamma rays produce electron and positron pairs and the positrons annihilate in two gamma rays. The kinetic energy of a photo electron produced by the photoelectric interaction is proportional to the energy of gamma rays. The number of electrons produced and the intensity of the gamma-ray are proportional to the concentration of the isotope that exists in the sample matrix. Therefore, the specific gamma-rays emitted from the material are used to identify and determine the concentration of an element present in the target material.

Figure 3.1 Various types of gamma ray interactions in a detector



A reaction between an incoming neutron and an atomic nucleus is called an "induced nuclear reaction". As a result of a nuclear reaction, one can form a new nucleus or excite the original nucleus. Various nuclear reactions can occur depending on the type and energy of interacting particles and the type and size of the target nucleus. Interaction of the neutron with the target nuclide and the nuclear reaction is shown below.



Target + neutron \rightarrow Possible reactions

As mentioned above, one neutron can induce many different reactions as the energy of the neutron changes. Therefore, it is rather critical to characterize as many parameters as

possible before inducing any nuclear reaction. Thermal neutrons ($E_n = 0.025\text{eV}$) are used in order to produce an (n, γ) reaction. As the energy of neutron increases, the probability of other reactions increases.

When a sample is irradiated with thermal neutrons, a small portions of the isotopes present in the matrix will capture the thermal neutrons in an amount proportional to their nuclear properties such as the thermal neutron capture cross section. The newly formed nucleus become excited and de-excite to ground state giving off their characteristic gamma-rays. The discrete gamma-ray energies along with the unique half-life of the radio nuclide make it possible to separate each element instrumentally. The amount of induced radioactivity produced for separate isotope at the end of irradiation and cooling can be calculated using the following equation:

$$A = N\phi\sigma(1 - e^{-\lambda t_i})e^{-\lambda t_c} \quad (3.2)$$

where A = Activity of the product radio nuclide in disintegration per second

N = Number of target nuclei present

ϕ = Neutron flux in neutrons per square centimeter per second.

σ = Activation cross section in barns (barn = 10^{-24}cm^2)

t_i = Irradiation time

t_c = Cooling time

λ = Decay constant ($\ln 2/t_{1/2}$)

$t_{1/2}$ = Half-life

The activity of an element of interest in a given matrix can be obtained using the equation (3.2). In a real experiment, the absolute value may changed due to the irradiation and counting geometry factors, neutron flux etc. In order to eliminate errors that may arise

from these variables, the comparative method is used. A standard material with an accurately known composition is irradiated along with the sample. Then using the equation below, the concentration of the element of interest can be calculated since all of the variables except the activity and the number of atoms present are unknown.

$$\frac{A_{sample}}{A_{standard}} = \frac{N_{sample}(e^{-\lambda t_c})_{sample}}{N_{standard}(e^{-\lambda t_c})_{standard}} \quad (3.3)$$

Since

$$N = \frac{\text{Wt. of element} \times \text{abundance}}{\text{Atomic Wt.}} \times \text{Avogadro's Number} \quad (3.4)$$

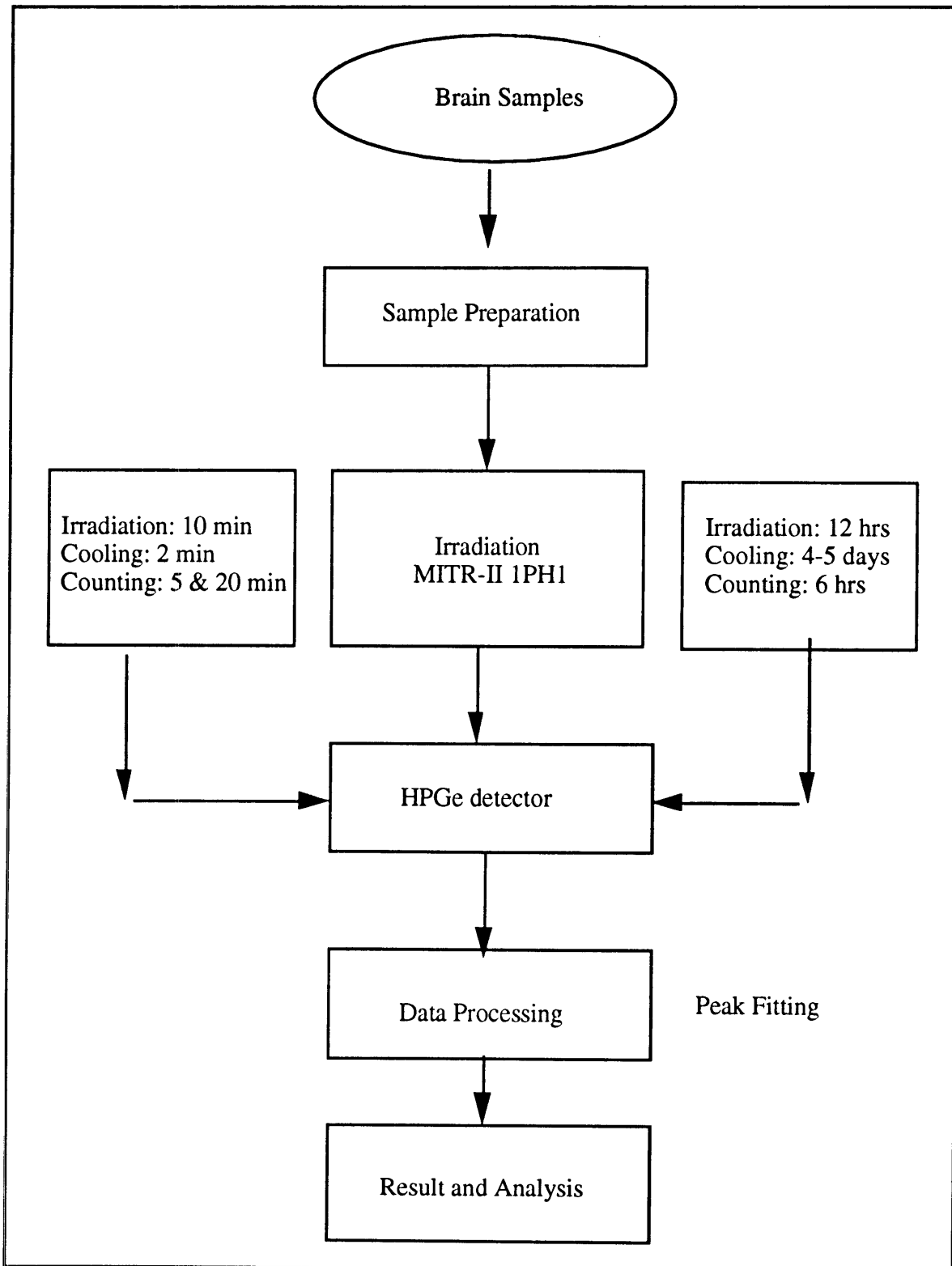
then

$$\text{Weight. in sample} = \frac{A_{sample}}{A_{standard}} \times \text{Wt. in standard} \quad (3.5)$$

The total activity observed from a sample after irradiation is the sum of all the gamma-rays emitted from radioisotopes. Since the gamma-rays are independent of one another, they can in principle, be resolved from each other, and Eq. (3.5) can be applied for every individual radioisotope present in the matrix.

3.2 Methodology (INAA)

In order to perform an instrumental neutron activation analysis (INAA), three parts should be comprised: a clean sample handling system, a suitable flux and energy of neutrons, sensitive counting system. A typical schematic diagram of INAA is shown in Figure 3.2.

Figure 3.2. Schematic diagram of the INAA

Similar to other nuclear techniques, sample preparation and handling are very important in INAA because the amount of element in sample is very small (in the range of 10 ug to 10 ng) (Olmez, 1989). In order to avoid sample contamination, samples must be handled in a clean environment, and in a container that does not contain any elements of interest. The most commonly used material is polyethylene, since it is cheap, and its interaction with thermal neutrons is very weak. The disadvantage of this material is that it can not stand very high temperature (Parry, 1991).

As mentioned in the previous section, (n, γ) reactions are induced by thermal neutrons. Thermal neutrons can be obtained from a neutron generator, radioisotopes, or a nuclear reactor. Nuclear reactors are the main sources of thermal neutrons because of their high neutron flux. Individually packaged samples are placed into a sample carrier and transferred into neutron sources for irradiation. The important conditions that may be altered to enhance the sensitivity of the analysis are the nature of the neutron flux and the length of irradiation. Irradiations are usually performed twice for short and long lived isotopes. Since the control of the thermal neutron flux in a reactor is not easy, the length of irradiation which is attainable in practice will depend on the way in which the reactor is operated. Two different types of irradiations, short and long, are usually performed for short-lived radioisotopes and long lived radioisotopes respectively. For short irradiation, samples are exposed to a neutron flux for several minutes and counted to observe radioisotopes with half lives ranging from 2.2-min Al-28 to 15h Na-24. Before the irradiation for long-lived radioisotopes, samples are cooled for several days so that the activity due to short-lived radionuclides reduces to near zero. Long irradiation is performed for several hours to produce activities in long-lived radioisotopes. After several hours to days of cooling, gamma rays are counted for several hours.

Two types of radiation detectors are generally used; the scintillation counter and semiconductor. When low activities are present in samples, the scintillation counter is

suitable because the efficiency of the semi-conductor is low. However, semiconductors are more commonly used since the resolution is very good.

The intensity of the characteristic emission from the irradiated sample are registered as a counting rate in the detector. The output of the signal is converted into digital by an analog-digital converter and saved in computer. The appropriate assessment and computation is performed by computer.

3.3 Detection Limits

One of the major differences between nuclear analytical techniques and other analytical methodologies is the ability to change parameters that affect the sensitivity so as to obtain the best achievable detection limits. It is desired that this should be accomplished without including any additional chemical steps such as separation or pre-concentration. The activity in equation (3.2), which is directly proportional to the sensitivity of the technique for a specific element, is a direct function of flux, cross section, number of atoms, irradiation decay, and counting times. Among these, flux is the most important parameter influencing the activity produced. In every research reactor there are several sample irradiation positions with different neutron fluxes available. Depending on the sample characteristics and expected concentrations of the element interest, different neutron fluxes can be used to produce sufficient activity for high quality and quantitative measurements. The activity produced at the end of an irradiation would also depends on the irradiation duration. Only 50% of the theoretically achievable activity will be produced after the sample is irradiated for one half-life of the expected radio nuclides. If it is economically feasible, irradiation time can be increased to produce maximum activity. This is applicable especially in the case of short half-lived isotopes, i.e. those with half-lives in the range of minutes to days.

In most cases there is no limit on the size of sample. Although optimum sample weight is 10-100 mg, it can be as small as micrograms, or as large as kilograms. Therefore, if suitable reference materials are used, and constant counting geometry between unknowns and elemental monitors are achieved, lower detection limits for an element can be obtained simply by increasing the sample size by orders of magnitude.

Another way of improving the detection capability is to increase the counting time. This also improve the overall counting statistics. However, the researcher must ensure that the necessary corrections should be made to account for the changes in dead-time during the counting duration. This is especially critical for the short half-lived isotopes during extended counting times.

3.4 Advantages and Disadvantages of nuclear analytical techniques

Advantages and disadvantages of INAA have been discussed extensively (Olmez, 1989), therefore, only a brief summary will be presented here. If interference-free detection limits are calculated for each element, one can obtain small numbers, about 10^{-12} g or less, for many elements. In a real sample, the limit of delectability of a particular element depends on the presence and amount of other elements in the matrix. However, the sensitivity for a wide range of elements is high compared with other analytical techniques.

There are other instrumental analysis techniques such as x-ray fluorescence (XRF), scanning electron microscopy coupled with XRF, secondary-ion mass spectrometry, and Auger spectroscopy available for elemental analysis. These techniques have many powerful applications, but they cannot accurately handle samples that are thick or in homogeneous.

Since there is no need to dissolve or change the physical forms of the samples, nuclear methods are fairly non-destructive. Also, after the activities have decayed to low

enough levels, samples can be re-analyzed using other analytical techniques except, in some cases where samples are damaged by radiation during long irradiation.

It is possible to determine concentrations of 20 to 40 elements in individual samples weighting 10 to 100 mg using nuclear analytical technique. This is rather important in cases where the sample size is limited.

While there are the many advantages, there are also disadvantages of this technique. The major disadvantage is that the equipment required is expensive and the number of people skilled in its application is so small that most potential users do not have access to these methods. Another disadvantage is the turn-around times for the analysis of the long lived isotopes are around 2-3 weeks because of the decaying time of the short-lived isotopes. This technique does not give information about the chemical composition of the sample since it is an elemental technique. Several elements such as Si, Ni, Pb and all elements lighter than Na can be determined marginally or are impossible to observe by INAA. Therefore, it should be supplemented by other analytical techniques for the determination of these elements.

3.5 Brief Review of the INAA at MIT-II

The MITR-II reactor is operated at 5MW producing neutron flux ranging from 1×10^{12} to $5 \times 10^{13} n/cm^2 sec$. Depending on the sample characteristics and expected concentrations of elements of interest, the researcher can choose irradiation locations to produce sufficient activity for quantitative measurements. The reactor operates routinely around the clock for 90-100 hours per week. For INAA, samples are transferred to MITR-II through a pneumatic tube facility and irradiated. The special feature of the sample irradiation facility in the MITR-II is that there is cooling system in the beam port. Thus, sample irradiation is always performed under ambient temperature conditions and material

loss from the sample heating can be minimized. There are three major rooms in INAA facility: the clean room, hot lab, and counting room. In the clean room, samples are packaged, usually with polyethylene bags, and transferred to the hotlab for irradiation where the pneumatic tube facility is located. After irradiation, these samples are repackaged with unirradiated clean bags and transferred to counting room where the high purity germanium (HPGe) detectors, multichannel analyzer (8192-channel pulse-height analyzers), and computer system are located.

For the short irradiation, samples, standard (Fly Ash) and reference (Orchard Leaves) material are exposed for 10 minutes of irradiation, 2 minutes of cooling, and counted for 5 and 20 minutes to collect spectrum for short lived elements. Long irradiation is performed after the samples are allowed to decay for several days. Samples are irradiated for 12 hours to build up long lived activities, cooled for several days, and counted for more than 6 hours. The spectra are analyzed using computer programs to search for the gamma-peaks for each isotope and the concentrations are determined on the basis of the relative area between the standard material (flyash) and the sample. In order to confirm the result, Orchard Leaves is used as a reference material.

Chapter 4 INAA of ALS and Control Samples

The methodology used in this study is similar to that published by Olmez (1989). The schematic diagram of this procedure is shown in a previous chapter (Figure 3.2).

4.1 Sample Preparation

Degenerated and healthy motor cortex tissue, cerebellum tissue and spinal cord fluid of patients with ALS were obtained from Massachusetts General Hospital (MGH). The descriptions of all samples and as well as dry-weights are given in Table 4.1. A total of 42 ALS samples (19 from motor cortex, 13 from cerebellum, 10 from spinal cord) and 29 Control samples (16 from motor cortex, 9 from cerebellum, 4 spinal cord) were examined.

Due to the very low level of many of the elements of interest and the small size of the samples available, extreme care was needed to prevent contamination. Samples were freeze-dried for two to three days to remove any water content from the samples. After the freeze drying, samples were weighted in order to obtain their dry weight. The dry weights of each sample and sample information is also given in Table 4-1.

The samples and standards were transferred into acid-cleaned polyethylene bags, heat sealed, individually labeled, and placed into a sample carrier. The schematic diagram of sample carrier is shown in Figure 4.2. In sample carrier, the samples were fixed with two pieces of polyfoam to prevent movement while the samples are irradiated. This was

Table 4.1. Descriptions and Dry-weights of all samples

Sample No	Dry-Weight (g)	Description a = ALS Motor Cortex
AM1	0.01055	a
AM2	0.01810	a
AM3	0.02464	a
AM4	0.01537	a
AM5	0.01231	a
AM6	0.01070	a
AM7	0.01055	a
AM8	0.02546	a
AM9	0.01291	a
AM10	0.02338	a
AM11	0.01404	a
AM12	0.01070	a
AM13	0.02252	a
AM14	0.02853	a
AM15	0.02118	a
AM16	0.0427	a
AM17	0.06083	a
AM18	0.03731	a
AM19	0.02702	a

Table 4.1. (Continued) Descriptions and Dry-weights of all samples

Sample No	Dry-Weight (g)	Description b = Control Motor Cortex
CM1	0.05059	b
CM2	0.01933	b
CM3	0.03445	b
CM4	0.03445	b
CM5	0.02622	b
CM6	0.02274	b
CM7	0.01688	b
CM8	0.03828	b
CM9	0.01864	b
CM10	0.01490	b
CM11	0.01781	b
CM12	0.01793	b
CM13	0.02014	b
CM14	0.03128	b
CM15	0.01209	b
CM16	0.02049	b

Table 4.1. (Continued) Descriptions and Dry-weights of all samples

Sample No	Dry-Weight (g)	Description c = ALS Cerebellum d = Control Cerebellum e = ALS Spinal Cord f = Control Spinal Cord
ACB1	0.01410	c
ACB2	0.02323	c
ACB3	0.02198	c
ACB4	0.02134	c
ACB5	0.01742	c
ACB6	0.01482	c
ACB7	0.03198	c
ACB8	0.02233	c
ACB9	0.01079	c
ACB10	0.01244	c
ACB11	0.01797	c
ACB12	0.01426	c
ACB13	0.01271	c
CCB1	0.02073	d
CCB2	0.02720	d
CCB3	0.02774	d
CCB4	0.01855	d

Table 4.1. (Continued) Descriptions and Dry-weights of all samples

Sample No	Dry-Weight (g)	Description c = ALS Cerebellum d = Control Cerebellum e = ALS Spinal Cord f = Control Spinal Cord
CCB5	0.01644	d
CCB6	0.02205	d
CCB7	0.05740	d
CCB8	0.03639	d
CCB9	0.02935	d
ASC1	0.01073	e
ASC2	0.07200	e
ASC3	0.01230	e
ASC4	0.00639	e
ASC5	0.02729	e
ASC6	0.04173	e
ASC7	0.06366	e
ASC8	0.05100	e
ASC9	0.02508	e
ASC10	0.01799	e
CSC1	0.02654	f
CSC2	0.02308	f
CSC3	0.01609	f
CSC4	0.03938	f

also needed to keep the geometry the same for each sample. All handling was performed in a clean-hood using Teflon tweezers prevent contamination.

4.2 Calibration and quality assurance/quality control (QA/QC)

The use of a standard material provides the best method of standardization in a quantitative analysis. It is very important to use the proper standard material to obtain the desired results. Fly Ash (NBS's SRM-1633a) was used as a multielement standard material in this study. In the preparation of samples and standard material for irradiation, care was taken to reproduce the geometry of the sample and ensure that the flux is also reproducible or can be corrected.

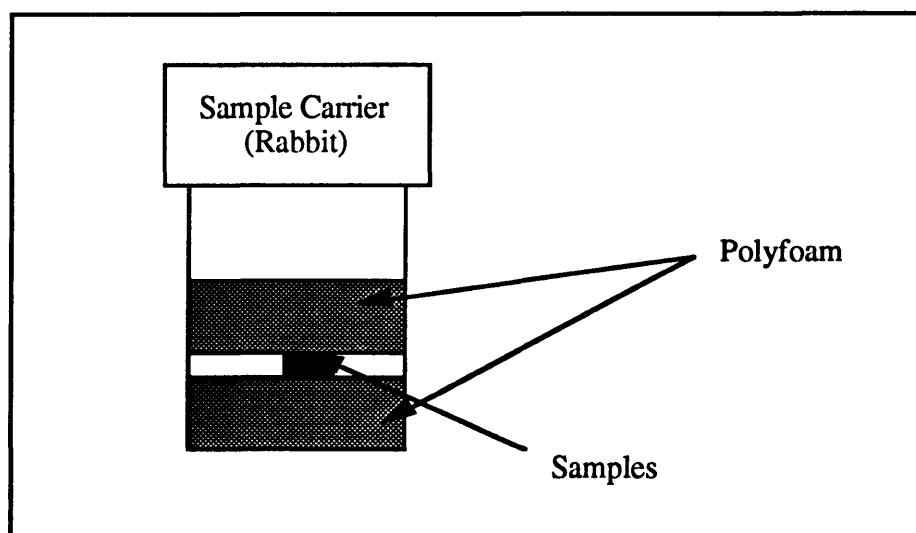
Analysis of a reference material with recommended values for the concentrations of the elements of the interest is one of the most valuable ways of checking the validity of the results of an experiment. Therefore, it is very important to have well analyzed reference standards than to match the matrix and trace element composition exactly. Blood, bone, muscle, and liver materials are usually used as a standard material in biological studies. In this study, Orchard leaves (NBS's SRM-1571) was used as a reference material.

4.3 Reactor Irradiation and Spectrum Collection

Irradiation was performed at a thermal energy neutron flux of 8×10^{12} n/cm² sec using the 1PH1 beam port. Samples were irradiated at the MITII reactor twice. First, they were irradiated for 5 minutes, cooled for 2 minutes and counted for 5 minutes to observe gamma-rays emitted from radioisotopes with short half-lives such as Mg, Al, Ti, V, Cl, K, Mn, Na. A pneumatic tube facility was used to shorten the sample transfer time. Samples were exposed to second irradiation for 12 hours after the samples were allowed to decay

for several days, cooled for several days. After the second irradiations, the samples were counted for over 6 hours to collect spectrum for long lived radioisotopes such as Sb, Br, Cd, Na, Cs, Cr, Eu, Fe, Rb, Se, and Zn.

Figure 4.1. Schematic diagram of the Sample Carrier (Rabbit)



The irradiated samples were repackaged in acid- cleaned polyethylene bags, placed into a lead container (pig), and brought into counting room. The samples were placed on the HPGe detector (Canberra, CT) to obtain the activity of samples. The counting geometry for samples and standard vials were maintained as identical as possible since the sample activity is strongly dependent on geometry. As shown in Figure 4.2, gamma rays emitted from the irradiated samples were fed into an amplifier through a preamplifier. The subsequent output signal from the amplifier was then fed into an one of the 8192-channel pulse-height analyzers (Canberra, CT). The analog signal was converted by the A/D converter.

The energy resolution and efficiencies of the detectors were measured using a NIST certified mixed radio nuclides point source (SRM 4275-109). The energy resolution and efficiency of this system was found to be 0.17% and 2.59% at 5.5 cm, at 100 keV, respectively. Energy calibration of the multichannel analyzer was performed by using Co-60 and Ba-133 sources. A plot of the calibration is shown in Figure 4.3. From the figure, the energy calibration was found to be

$$\text{Energy (keV)} = 0.437 \times \text{Channel No.} + 11.2$$

The obtained spectra were saved in the computer (ND 9900 Genie system run on VMS 200) for the calculation of elemental concentration, and were ready for peak fitting to determine the concentration of elements.

Figure 4.2 Schematic diagram of the electronics of spectroscopic system

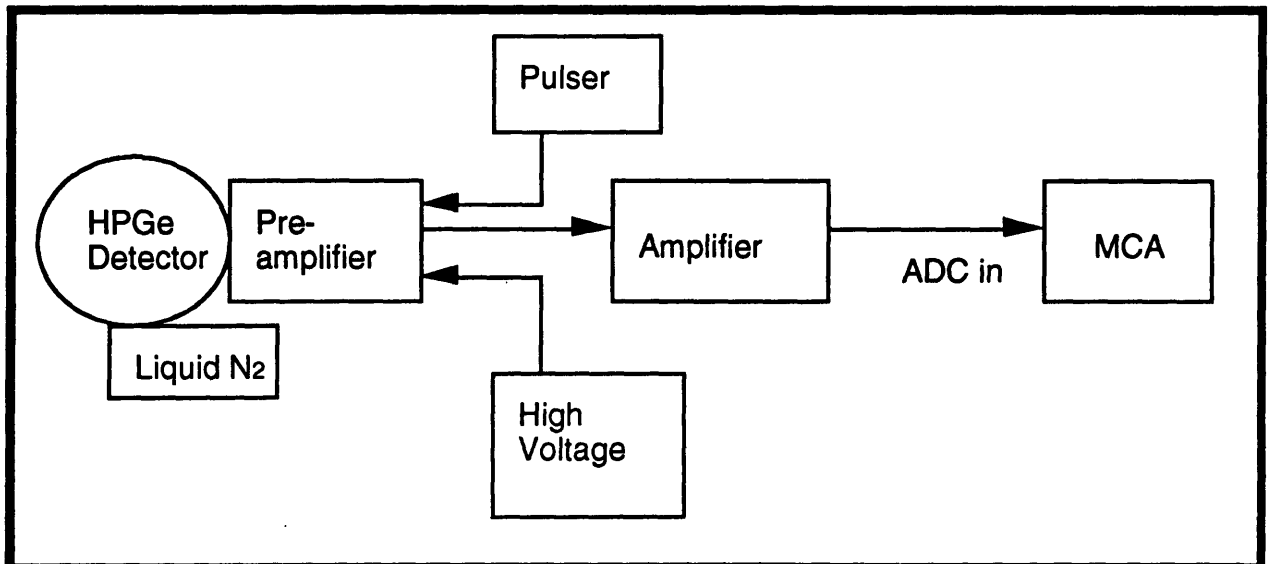
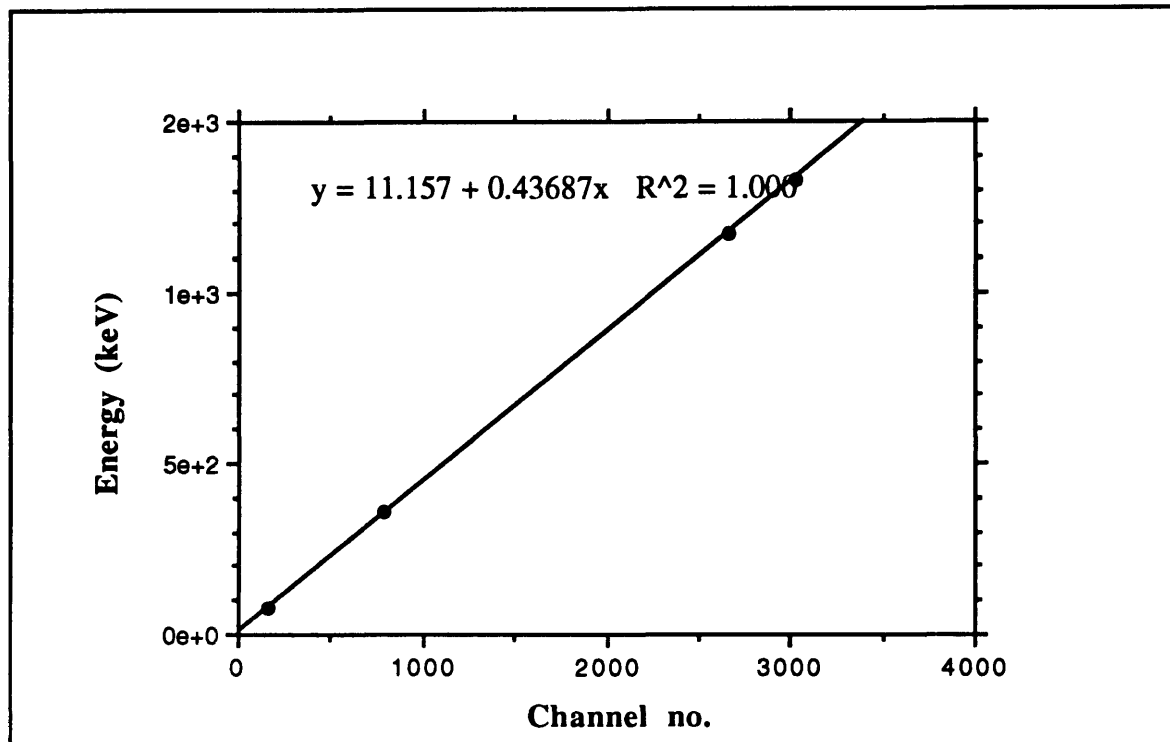


Figure 4.3 Energy Calibration curve with Co-60 and Ba-133 sources for the HPGe detector



4.4 Determination of Trace and Minor Element in Samples

Due to the different types of interactions of gamma rays with the detector material, many peaks were found for each element. In order to best find radionuclides, the gamma rays, irradiation time, decay times, and counting times, for each element were selectively determined. The gamma transition energy of each element used to determine the elemental concentration, along with the elemental composition of standard and reference are shown in Table 4.2. Magnesium, Al, Ti, and V were obtained from 10 minutes of irradiation and 5 minutes of counting. Most of the peak area was fitted automatically by the computer-directed program (VMS 200) to search for the best-fit-gamma energy peak for each isotope. There were some peaks that the computer could not resolve because the peaks were very close together. These peaks were fitted manually.

A comparison method was used to determine the concentration of each element in the sample matrix. The elemental information of standard materials have been stored in library files. These libraries were used to compare the peak area between the sample and standard, and used to calculate the elemental concentration of unknown samples. The comparison of the concentrations of reference element, given by NIST along with the measured values, are given in Table 4.2. Among the many observed elements, only eighteen elements were analyzed. The hard copy of the elemental concentration of each sample along with detection limits is given in Appendix. Any possible interference and criteria used in the determination of elements are listed below.

Magnesium was determined from 843.8 keV energy peaks. This peak was located very close to the manganese peak (846 keV). Therefore, the peak area of the two peaks in standard material and samples were fitted manually because the computer could not resolve them. In order to verify the fitted peak area, the concentrations of magnesium was compared with the values given from NIST.

It was difficult to determine the concentration of aluminum in samples due to the low concentration, very short half life, and other factors, although the peak area for aluminum in the standard material was very high.

Chlorine concentration was not included in the standard material, and was very low, making the results questionable. In order to determine the concentration of chlorine in samples a reference material was used. One of the values of chlorine in Orchard Leaves determined by the computer is shown in Table 4.2. The chlorine concentration obtained in this study was five times less than the values given by NIST. In order to correct this error, the peak area of chlorine in the reference and samples were compared. The equation used to determine the concentration of chlorine is

$$C_s = \frac{C_r}{C_{r'}} \times C_{s'} \quad (4.1)$$

where C_s is the concentration of Cl in sample

$C_{s'}$ is the concentration of Cl obtained from experiment in sample.

C_r is the concentration of Cl in Orchard leaves (690 ppm)

$C_{r'}$ is the concentration of Cl obtained from the experiment using Orchard leaves.

Chlorine values for sample CCB7, CCB8, CCB9, ASC1, ASC2, ASC3, ASC4, ASC5, ASC6, ASC7, ASC8, ASC9, ASC10, CSC1, CSC2, CSC3, CSC4 were not corrected due to the missing spectra of the reference material.

Potassium and sodium values were calculated by averaging the two values obtained from the short and long experiments. The variation between the two measurement was less than 15%. For some samples (CCB7, ASC9, and CSC2) that had high dead time in short experiments, the concentrations of potassium and sodium were taken from the Long irradiation results. The high level of Na obtained by experiments results from the Na content in bag. Therefore, this is not our problem.

Table 4.2 Comparison of elemental concentration between the reference and measured values.

Element	SRM-1633a Fly Ash	SRM-1571 Orchard Leaves	Measured Values (Orchard Leaves)	Energy (keV)
Mg ^a (%)	0.455 ± 0.010	0.62 ± 0.02	0.64	843.8
Al ^a (%)	14.0 ± 1.0	---	0.043	1779.0
Ti ^a (%)	0.8 ± 0.1	---	0.0032	320.1
V ^a (ppm)	300 ± 50	---	0.0013	1434.2
Cl ^b (ppm)	---	690	136	1642.0
K ^b (%)	1.88 ± 0.08	1.47 ± 0.03	1.21	1525.0
Mn ^b (ppm)	190 ± 10	91 ± 4	83	846.6
Sb ^c (ppm)	6.15 ± 0.15	2.9 ± 0.3	3.1	546.0
Br ^c (ppm)	2.31 ± 0.16	10.	10	554.3
Na ^c (%)	0.165 ± 0.004	0.0082 ± 0.0006	0.024	1368.5
Cr ^c (ppm)	193 ± 5	2.6 ± 0.3	3.4	320.0
Eu ^c (ppm)	3.50 ± 0.04	---	---	964.0
Fe ^c (%)	9.51 ± 0.18	0.0300 ± 0.0020	0.028	1291.6
Rb ^c (ppm)	134 ± 4	12 ± 1	12	1076.6
Se ^c (ppm)	10.3 ± 0.6	0.08 ± 0.01	0.44	264.5
Zn ^c (ppm)	220 ± 10	25 ± 3	45	1115.5

a = Irradiation: 5 minutes, Counting: 6 minutes

b = Irradiation: 5 minutes, Counting: 20 minutes

c = Long Irradiation

Chapter 5 Statistical Analysis

5.1 Summary Statistics

A computer program (Statgraphics Plus 6.0, Manugistics) was used to calculate the statistical values such as average, standard deviation, range, and standardized Kurtosis coefficient in order to analyze the quantitative variables of the data sets. The calculations used in the program have been given by Johnson and Wichern (1982). The average represents the central tendency of the data. In order to compare the concentrations of elements between ALS and control samples, average values were calculated.

The standard deviation, variance, and range measure the spread or dispersion of the data sets. Among these parameters, standard deviation was calculated to estimate the uncertainty of these data. The Kurtosis coefficient reveals how flat or steep the distribution of the data is with respect to a Gaussian or normal distribution. For a normal distribution, the Kurtosis coefficient is zero. When the coefficient is less than zero, the curve is flat with short tails. When the coefficient is greater than zero, the curve is either very steep at the center or has relatively long tails.

The standardized Kurtosis coefficients examine the significant deviation from the normal distribution. When a large sample is used, the standardized Kurtosis coefficient is approximately unit normal. When the values for the standardized coefficients are outside the range -2.9 to +2.0, the data may depart significantly from a normal distribution.

In order to compare the concentrations of elements in brain tissue obtained in this study with literature value, the average values of the motor cortex and cerebellum in control

were used. Table 5.1, shows a comparison of the elemental concentrations in control brain samples with values listed by Z. Guisun et al.(1991). The data from this study was within the range of the standard deviations.

Table 5.1 Analytical results of measured elemental concentrations in control brain and comparison with literature values ($\mu\text{g/g}$ dry weight).

Elements	Present study $\mu\text{g/g}$ dry weight (n)	Z. Guisun et al.(1991) $\mu\text{g/g}$ dry weight (n=23)
Mg	607 \pm 167(22)	700 \pm 101
Al	5.6 \pm 5.7(12)	
Ti	9.0 \pm 5.0 (7)	
V	0.01 \pm 0.01 (3)	
Cl	6700 \pm 1800 (20)	6420 \pm 831
K	11000 \pm 2700 (25)	13100 \pm 1500
Mn	1.3 \pm 0.54 (23)	1.16 \pm 0.43
Sb	0.09 \pm 0.18 (12)	
Br	5.1 \pm 3.5 (23)	3.27 \pm 1.16
Cd	0.15 \pm 0.12 (7)	
Na	6500 \pm 2000 (24)	6160 \pm 733
Cs	0.09 \pm 0.07 (17)	
Cr	1.8 \pm 1.5 (24)	0.80 \pm 0.53
Eu	0.02 \pm 0.01(14)	
Fe	280 \pm 95 (24)	224 \pm 35
Rb	11 \pm 4.4 (24)	22.2 \pm 7.0
Se	0.86 \pm 0.62 (24)	0.57 \pm 0.17
Zn	37 \pm 12 (24)	41.3 \pm 5.6

5.1.1 Motor cortex

In Tables 5.2 and 5.3, the average, standard deviation, range, number of samples and standardized Kurtosis coefficient (SKC) of each element are shown in the motor cortex of ALS and the control samples. In calculating the averages, the Br value of AM2, Ti value of AM7, and Mg value of CM16 were excluded because the concentrations of these elements were over 50 % higher than the others.

Standardized Kurtosis Coefficient (SKC) values indicate that the distributions of Br, Cs, Cr, and Eu in ALS and Al, Sb, and Ti in the control samples depart significantly from a normal distribution. None of the elements is known as essential for animal life except Cr. It has been known that nonessential elements show a wider variation than essential elements. Even so, the elements having high SKC values known to be a nonessential need to be studied further because these elements might some time later be defined as essential. Chromium is one of the essential trace elements that poses the greatest degree of technical difficulty in biochemical analysis. The nutrition requirement of this element in humans has not been established. But the low levels of Cr in blood and tissues of the elderly, as well as diabetics, and the toxicity manifested with skin rashes have been known. The significance of this observation is not known at this time. From the above statements, the high SKC value of Cr in ALS samples may be explained as being a secondary or causative factor for ALS.

The ratios of average values in the motor cortex of ALS and the control samples are compared in Figure 5.1. More than 10 % higher values of Mg, Al, V, Sb, Cs, Cr, Eu, Fe, and Se were observed in ALS as compared to the control samples. In ALS samples, the levels of Ti, Br, Cd, and Rb were lower than in the control. No significant differences were found for Cl, K, Mn, Na, and Zn.

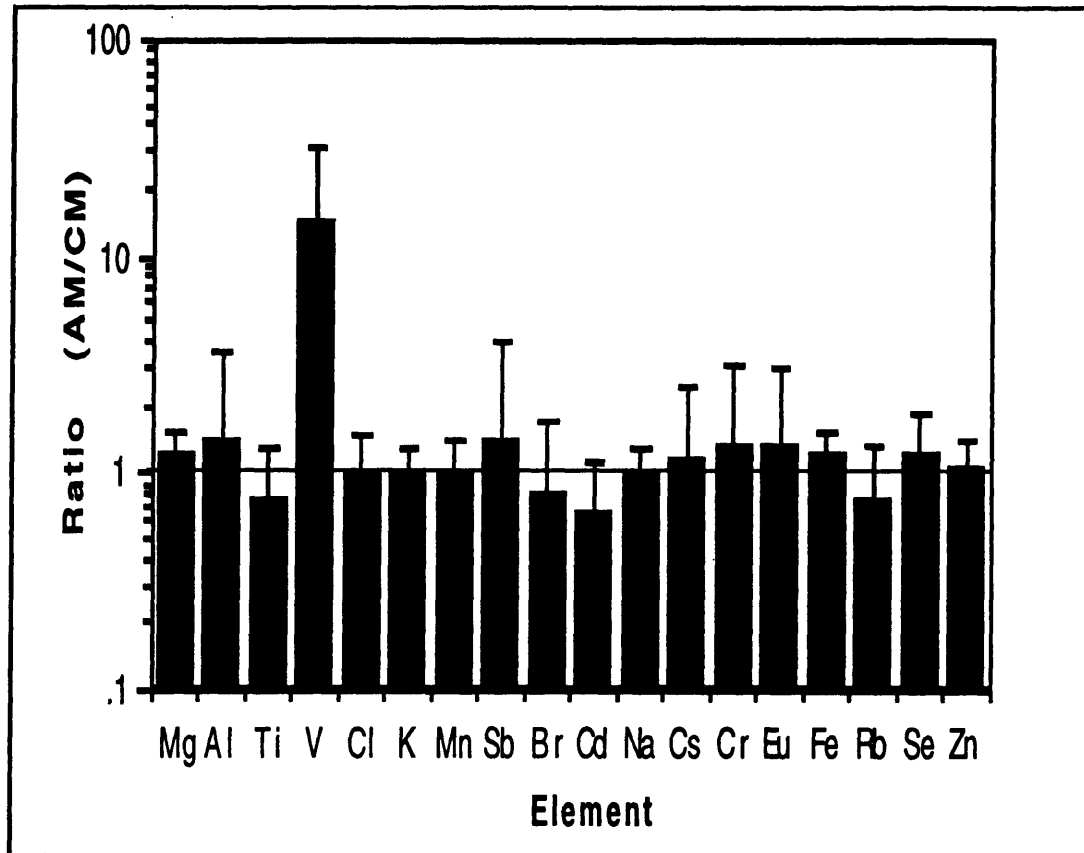
Table 5.2 Average, Standard deviation, Range, number of samples and Standardized Kurtosis coefficients in the motor cortex ALS samples ($\mu\text{g/g}$ dry weight basis)

Element	Sample size	Average ($\mu\text{g/g}$)	SD ($\mu\text{g/g}$)	Range ($\mu\text{g/g}$)	SKC
Mg	19	692	143	529 - 976	-0.23
Al	12	7.1	7.1	0.74 - 22	1.11
Ti	6	7.6	2.4	3.5-9.9	1.38
V	3	0.15	0.093	0.075-0.25	---
Cl	19	6700	2100	4600-13000	1.70
K	19	11000	2500	6900-17000	0.57
Mn	19	1.2	0.39	0.64-1.7	-1.35
Sb	11	0.19	0.20	0.025-0.57	0.033
Br	17	4.9	4.5	0.98-20	6.8
Cd	6	0.13	0.059	0.042-0.19	-1.28
Na	19	7100	1900	4300-12100	1.08
Cs	11	0.11	0.095	0.045-0.37	4.10
Cr	19	1.9	2.2	0.11-9.7	8.9
Eu	10	0.031	0.030	0.009-0.11	3.2
Fe	19	364	78	274-510	-0.8
Rb	18	7.9	5.6	1.1-21	0.43
Se	19	0.93	0.35	0.4-1.5	-0.95
Zn	19	39	8.1	26-55	-0.76

Table 5.3 Average, Standard deviation, Range, number of samples and Standardize Kurtosis coefficients in the motor cortex control samples.

Element	Sample Size	Average ($\mu\text{g/g}$)	SD ($\mu\text{g/g}$)	Range ($\mu\text{g/g}$)	SKC
Mg	13	560	75	470-710	-0.50
Al	8	4.9	5.9	1.1-19	3.84
Ti	5	9.7	5.2	5.1-17	-0.66
V	3	0.014	0.0082	0.006-0.022	---
Cl	14	6810	2100	3600-11000	0.10
K	16	11000	1000	8500-13000	0.17
Mn	14	1.1	0.38	0.56-1.9	-0.47
Sb	8	0.13	0.21	0.023-0.63	3.65
Br	15	6.1	3.7	1.2-13	-0.51
Cd	5	0.20	0.11	0.1-0.35	-0.55
Na	15	7400	1700	4100-11000	-0.10
Cs	12	0.11	0.079	0.03-0.3	1.74
Cr	16	1.3	0.83	0.39-3.3	0.47
Eu	11	0.022	0.014	0.005-0.048	-0.306
Fe	16	302	70	213-405	-1.27
Rb	16	10	3.8	5.88-17	-0.80
Se	16	0.75	0.24	0.3-1.2	-0.26
Zn	16	37	9.9	22-55	-0.10

Figure 5.1. Plot of the ratios of the average concentrations of ALS and the control samples taken from motor cortex tissue



5.1.2 Cerebellum

A total of twenty two samples of cerebellum tissue (thirteen from ALS and nine from the control) were analyzed. Tables 5.4 and 5.5 show the average, standard deviation, range, and SKC of elements in the cerebellum of the ALS and the control samples, respectively. The values for Ti, V, Cd, and Eu were obtained from fewer than five samples. Since those elements showed high uncertainty in determination of peak area due to high background, interference from other gamma energy peak and low concentration of the element, the ratios of these elements were not compared.

Table 5.4 Average, Standard deviation, Range, number of samples and Standardized Kurtosis coefficients in the ALS samples taken from the cerebellum.

Element	Sample Size	Average ($\mu\text{g/g}$)	SD ($\mu\text{g/g}$)	Range ($\mu\text{g/g}$)	SKC
Mg	13	823	122	595-990	-0.54
Al	6	9.67	9.66	1.8-26	0.10
Ti	2	13.5	3.53	11-16	---
V	3	0.14	0.11	0.054-0.26	---
Cl	13	6948	2208	3340-11650	0.511
K	13	14500	2710	7390-18000	2.44
Mn	13	1.88	0.40	0.79-2.4	3.49
Sb	6	0.032	0.030	0.01-0.09	2.14
Br	13	4.8	4.0	0.16-11	-1.20
Cd	1	0.027	---	---	---
Na	13	5930	1490	3500-8900	-0.081
Cs	13	0.13	0.072	0.033-0.27	-0.48
Cr	10	1.4	1.31	0.27-4.5	1.94
Eu	11	0.039	0.029	0.009-0.01	-0.14
Fe	13	290	88.1	177-495	1.38
Rb	12	9.3	6.12	1.5-22	0.16
Se	13	0.74	0.23	0.47-1.2	-0.49
Zn	13	37	7.3	27-54	1.18

Table 5.5 Average, Standard deviation, Range, number of samples and Standardized Kurtosis coefficients in the control samples taken from cerebellum.

Element	Sample Size	Average (ug/g)	SD (ug/g)	Range (ug/g)	SKC
Mg	9	674	238	130-960	2.17
Al	4	6.8	6.16	2-15	-0.24
Ti	2	7.2	5.4	3.3-11	---
Cl	6	6340	1100	4330-7530	1.29
K	9	12000	4400	2200-16200	1.422
Mn	9	1.5	0.67	0.85-2.7	-0.53
Sb	4	0.0075	0.0047	0.003-0.014	0.89
Br	8	3.3	2.3	1.1-7.4	-0.0048
Cd	2	0.031	0.017	0.019-0.043	---
Na	9	4911	1579	2900-7200	-0.86
Cs	5	0.066	0.043	0.02-0.11	-1.34
Cr	8	2.81	2.07	0.7-6.9	0.72
Eu	3	0.020	0.0085	0.011-0.028	---
Fe	8	235	127	25-414	-0.10
Rb	8	10.8	5.8	0.93-18	-0.051
Se	8	1.09	1.03	0.23-3.5	3.2
Zn	8	37	16.4	9.8-60	-0.018

Standardized Kurtosis Coefficients (SKC) indicate that the distribution of Mn in ALS samples and Se in the control samples are very different from the normal distribution. Both Mn and Se are essential trace elements for human life. The high value of Mn in ALS

may indicate that either abundance or deficiency would affect the formation of neurofibrillary in cerebellum tissue. The high variations of selenium in the control samples are not understood.

The average values of elements in ALS and the control samples were compared (Figure 5.2). High levels of Sb, Cs, and Eu in ALS samples were observed. This trend may be the result of the small number of samples, but it may also indicate some characteristic effects of these elements. There were low levels of Cr, Rb, and Se in the ALS samples. Chromium and Se are essential trace elements. There may be a deficient effects of these elements on cerebellum tissue of ALS patients

5.1.3 Spinal Cord

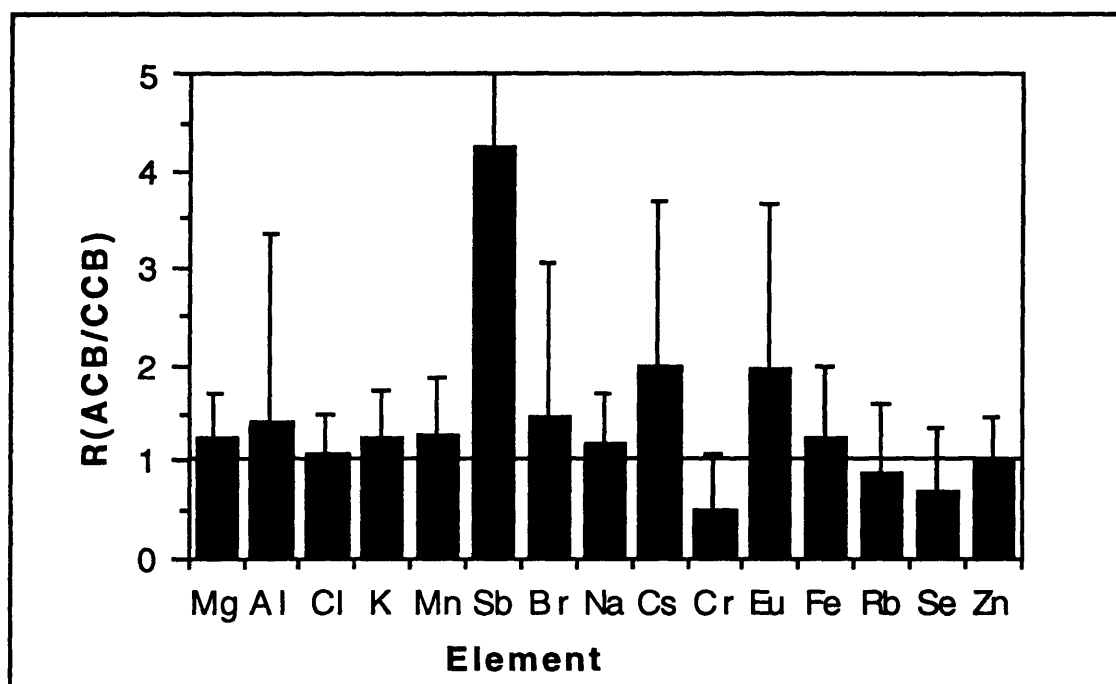
Summary statistics were applied to the data sets of the spinal cord samples. Sample numbers were small compared to the other tissue samples (ten samples from ALS and three from control samples). Therefore, the SKC values were not obtained for the control samples. Antimony, Br, and Se showed high deviations from a normal distribution. The average values of K and Fe in the spinal cord were much smaller than in the motor cortex and cerebellum tissue. Despite the small sample number analyzed, the elemental ratios of ALS and the control samples were compared (Figure 5.3). Different from the brain data, a lower level of Al was observed. The levels of Ti, Mn, Br, Cs, Cr, and Fe were higher in ALS than in the control samples. Similar to the cerebellum result, there were lower levels of Rb and Se. There were no significant differences in Mg, K, and Na.

5.1.4 Discussion

Magnesium is an essential element in human life which is involved in nerve impulse conduction. The toxic effects of magnesium on the central nervous system have been

known. The SKC test showed that in three different sites, the distributions are similar to the normal distribution. The levels of this element in ALS samples were also similar to the control samples (ratios were less than 25%). From the above result, it could be concluded that magnesium is probably not related to ALS.

Figure 5.2 Plot of the ratios of average concentrations of ALS and the control samples taken from cerebellum



A number of observations indicate that high concentrations of Al may be toxic to the nervous system. The toxicity of Al has also been described by Wisniewski et al (1980). Traub et al.(1981) determined brain Al levels in patients with degenerative CNS disorders and found that the concentrations of the element were not higher than the upper limit of normal (defined as 4 $\mu\text{g/g}$ of dry weight in 2 control and 1 ALS samples). Another study showed an elevated level of Al concentration in the spinal cord of ALS samples (Yoshimasu et al. 1981). In this study, the high level of Al was observed in the motor

cortex and cerebellum of ALS samples (4.9 $\mu\text{g/g}$ in 8 control and 8.4 $\mu\text{g/g}$ in 18 ALS samples). In the spinal cord, the Al level was less in ALS samples than control samples. The number of samples were four from ALS and two from the control. The opposite result in the spinal cord may be a result of the small number of samples. Many researchers have claimed that the Al accumulation in the brain has been a cause of the disease. On the other hand, some researchers reported that the Al accumulation of these patients occurs after extensive death of the nerve cells due to neurofibrillary tangle formation. In order to explain the effects of Al in ALS disease, further studies are necessary.

Concerning Ti a low level in the ALS samples taken from motor cortex, and a high level taken from spinal cord were found. These results are not significant because there was high uncertainty in determining the concentration of titanium. The role of Ti in this disease is unknown

Vanadium is one of the essential trace element in human life. The toxicity of this element is attributed to its ability to inhibit enzyme systems. However, the specific human requirement of this element is not established well. The concentration of V in man is 0.3 ppm and the average intake is 1000-4000 μg per day. The values of this element were analyzed only from the motor cortex samples found to be 0.15 and 0.01 $\mu\text{g/g}$ in the ALS and control samples respectively. Only three samples were analyzed for control, therefore the elemental levels of this elements are not compared here.

The ratios of Na and Cl in the ALS and control samples were close to one taken from motor cortex and cerebellum tissue. The SKC values showed that the distribution of these elements were similar to the normal distribution, indicating that the levels of Cl and Na in the ALS samples were normal. Both of these elements are essential elements in human life. An excess or deficiency of these elements causes characteristic diseases. Since there were no significant differences between the ALS and the control samples, these elements are not intensively studied here.

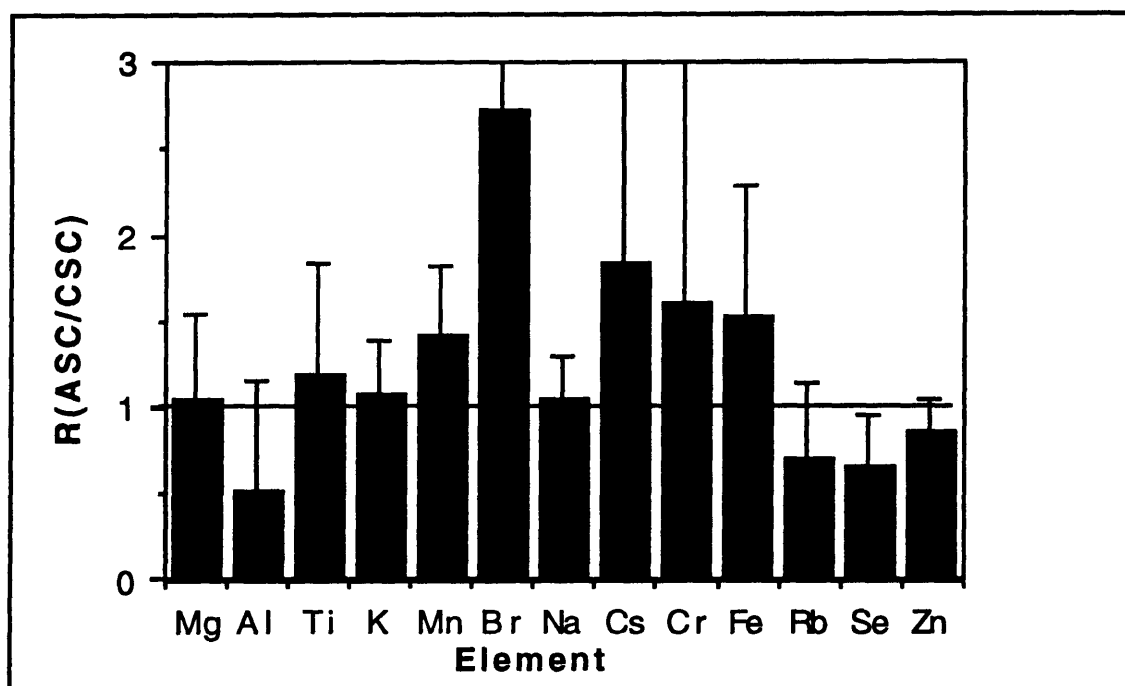
Table 5.6 Average, Standard deviation, Range, number of the samples and Standardized Kurtosis coefficients in the ALS samples taken from the spinal cords.

Element	Sample Size	Average ($\mu\text{g/g}$)	SD ($\mu\text{g/g}$)	Range ($\mu\text{g/g}$)	SKC
Mg	10	588	248	188-1076	0.48
Al	4	4.4	2.7	0.73-7.3	0.69
Ti	8	14.3	6.26	6.8-24	-0.39
V	6	0.22	0.25	0.03-0.58	-0.87
K	10	5600	1200	4000-8100	0.69
Mn	10	2.04	0.56	1.4-3	-0.67
Sb	7	0.019	0.019	0.07-0.062	3.28
Br	10	5.26	6.44	1.4-23	5.36
Cd	7	0.043	0.067	0.0037-0.19	3.3
Na	10	7240	1560	5500-9600	-0.84
Cs	9	0.12	0.069	0.038-0.27	1.43
Cr	10	5.57	6.28	0.93-19	1.0
Eu	7	0.043	0.036	0.002-0.095	-0.76
Fe	9	126	59	56-232	-0.060
Rb	7	6.27	3.9	2.4-12	-0.69
Se	8	0.58	0.23	0.4-1.1	2.85
Zn	10	24.2	4.49	18-32	-0.63

Table 5.7 Average, Standard deviation, Range, number of the samples and Standardized Kurtosis coefficients in the control samples taken from spinal cords .

Element	Sample Size	Average ($\mu\text{g/g}$)	SD ($\mu\text{g/g}$)	Range ($\mu\text{g/g}$)	SKC
Mg	3	565	130	488-715	---
Al	2	8.45	9.3	1.9-15	---
Ti	3	12.1	4.2	7.3-15	---
V	2	0.12	0	---	---
K	3	5200	810	4300-5900	---
Mn	3	1.43	0.058	1.4-1.5	---
Br	0.3	1.93	0.40	1.5-2.3	---
Cd	1	0.026	---	---	---
Na	3	6900	873	6200-7900	---
Cs	2	0.066	0.03	0.044-0.087	---
Cr	3	3.47	1.63	2.2-5.3	---
Fe	3	82.7	12.5	70-95	---
Rb	3	9.07	2.18	6.7-11	---
Se	3	0.88	0.22	0.67-1.1	---
Zn	3	28	3	25-31	---

Figure 5.3 Plot of the ratios of average concentrations of ALS and the control samples taken from spinal cord



Potassium is used by the body to regulate the osmotic pressure, and is involved in nerve impulse transmissions. The functions of this element have been established very well. Potassium in the samples showed normal distribution, and the concentrations of this element were in the same range for both the ALS and control samples. This suggests that this element does not play a causative role in ALS.

Manganese is one of the elements that has been suspected as a causative factor of ALS. There have been many studies on the level of Mn in ALS and high concentrations of Mn, Al, Fe, and Ca were found in southern Guam rivers by Zolan and Neils (1986). The high incidence of ALS in Guam could be related to the abundance of Al and Fe in drinking water since the intake of these elements is related to drinking water. Yase (1972) also analyzed samples by NAA in the spinal cord and brain of the people from the Kii Peninsula

and Japan where these elements are present in soil and water, and found no differences. In this study, no differences were observed in motor cortex tissue, which agrees with the previous study. However, elevated levels of Mn were observed in the cerebellum and spinal cord, and this agrees with the result obtained by Miyata et al. (1983). SKC values indicated that the distribution of manganese in the ALS samples taken from cerebellum tissue deviates from a normal distribution. This may explain the different result obtained from a different study, that both excessive and deficient participation of Mn content in tissue will affect the degeneration.

Even though Sb is one of the least studied elements, the role of this element is important in human metabolism. Therefore, the concentration of this element was compared in this study to provide information for further research. High concentrations of Sb were observed in the ALS samples taken from the motor cortex and cerebellum. There were high SKC values in the control samples taken from motor cortex, in the ALS samples taken from spinal cords. The significance of this trend is unclear.

The levels of Br were similar ALS and control samples taken from the motor cortex and spinal cord, but high in the ALS samples taken from cerebellum tissue. The distribution of this element in the ALS sample taken from motor cortex and the control samples taken from spinal cord fluid did not follow the normal distribution.

Chromium is also one of the essential trace elements that causes growth retardation when the concentration in the body is low. The toxicity usually manifests itself in skin rashes from industrial exposure. The concentrations of Cr were higher in ALS samples taken from motor cortex and spinal cord than in the control samples, but lower in ALS samples taken from cerebellum tissues. The reason for the opposite trends of the motor cortex and the cerebellum tissue is not understood. The distribution of Cr was wide in the ALS sample taken from motor cortex giving similar trends as Br. In order to examine any

underlying relations between these elements, correlation analysis and principal factor analysis were performed.

Iron is an essential element that is involved in oxygen transportation. High concentrations of this element in soil and water of Guam island have been observed. In the present study, the average value of Fe in all three parts of ALS samples were higher than in the control. The high concentration of iron in ALS samples may be related to accumulated blood cells in the degenerated area. Normal distributions were observed for Fe in all sample sites.

Little is known about the function of Rb in the human body. Rubidium is chemically similar to K and Cs. The relationships among K, Cs, and Rb will be discussed in the following section. Despite having chemical properties similar to K, the level of rubidium in the ALS samples were quite opposite from that of K. Although this observation was not explained here, there might be some effects of Rb on the process of neurofibillary formation.

Selenium is an element whose importance in humans has recently been established. The specific role of this element in the brain is unknown. In this study, high levels in the motor cortex and low levels in the cerebellum and spinal cord were observed. Zhwang G. et al.(1991) observed an elevated level of Se in tumor cells. The results obtained in this study along with previous studies may suggested that an abnormal content of Se affect body function. And this may be true for ALS case too.

Zinc is an essential trace element which is important in the physiological function of body since it is a component of several enzymes. It is known that the a deficiency of Zn during pregnancy results in congenital malformations in rats. And high concentrations of Zn in human tissue have been measured in skin malignancies. Although, Danielsen et al.(1969) observed lower concentrations of Zn in cancerous tissue, Zhuang G. et al. (1991) showed higher levels in the same kind of tissue. The present study, however, showed no

significant differences in the motor cortex or cerebellum, and only a low level in the spinal cord. The reason for this difference in concentration is not clear.

5.2 Correlation Analysis

The correlation analysis procedure generates a matrix of correlation coefficients for a set of observed values. This provides a preliminary view of the relationships among variables. The correlation analysis procedure is also used to eliminate redundant variables from factor analysis. If two elements are strongly correlated, only one element can be used as an independent variable. Correlation coefficients provide a normalized and scale free measure of the linear association between two variables. The coefficient values fall between +1 and -1. A positive correlation indicates that the elements vary in the same direction while a negative correlation indicates that the elements vary in the opposite direction. Statistically independent variables give an expected correlation of zero.

In present study, correlation analysis was performed to examine whether there is any relationships among trace and minor elements in ALS disease as well as in control samples. The sample correlation coefficients were obtained by multivariate analysis procedure (Statgraphic Plus 6.0, Manugestics).

The computer program computes the correlation coefficient for the two variables represented by the cell, the sample size, and the significance level of the correlation. A result is called insignificant if the significance level is less than 0.05, and highly significant if its significance level is less than 0.01.

The results of the correlation analysis are shown in Tables 5.8 and 5.9 for ALS brains and the control brains, respectively. Elements with a correlation coefficient below 0.05 were not included. Magnesium was positively correlated with Mn and zinc in control samples. In ALS samples, it was positively correlated with K and Mn and negatively correlated with Sb. Since Mg did not show any significant results in the comparison of

average values, the significance of this relationship is not discussed here. Sodium, Cl, Fe, Rb, and Zn were positively correlated with Br in the control samples. These relationships indicate a charge balance, since Na and Rb are alkali metals and Cl and Br are halogens. In order to balance the charge, Br must be correlated with Rb. In the ALS sample, there was no correlation between Br and Rb. This may indicate that a broken charge balance between Br and Rb in ALS disease may cause the degeneration of tissue, and low a level of Rb in brain tissue may be a precursor to ALS. As a summary, there were many correlation among the elements in control. Samples did not show many correlations in ALS indicating the possibility of unbalanced metabolism of trace and minor elements.

Correlation analyses of spinal cords were not performed since the number of samples analyzed were limited.

Table 5.8 Correlation Analysis data for ALS brains samples

Primary variable	Variables with statistically significant correlation($p \leq 0.05$) (+/- = Positively/Negatively correlated r, sample size, p)		
Mg	K (+0.52, 33, 0.002)	Mn (0.62, 33, 0.0001)	Sb (-0.52, 17, 0.032)
Al	Eu (0.70, 10, 0.025)		
Ti	---		
Cl	Br (0.36, 30, 0.049)	Na (0.87, 32, 0.0000)	Rb (0.40, 30, 0.028)
K	Mg (0.52, 33, 0.0020)	Mn (0.51, 33, 0.0027)	
Mn	Mg (0.62, 33, 0.0001)	K (0.51, 33, 0.0027)	
Sb	Mg (-0.52, 31, 0.033)	Eu (0.69, 9, 0.0389)	Fe (0.49, 17, 0.046)
Br	Cl (0.36, 30, 0.049)	Na (0.38, 31, 0.036)	
Cd	Zn (-0.72, 8, 0.044)		
Na	Cl (0.87, 32, 0.00)	Br (0.38, 31, 0.036)	Rb (0.42, 31, 0.018)
Cs	Sb (0.75, 11, 0.0085)	Eu (0.71, 22, 0.0002)	Rb (0.41, 23, 0.052)
Cr	---		
Fe	Sb (0.49, 17, 0.046)		
Rb	Na (0.42, 31, 0.018)	---	---
Se	---	---	---
Zn	Cd (-0.72, 8, 0.044)	Na (0.36, 33, 0.039)	Fe (0.38, 32, 0.030)

Table 5.9 Correlation Analysis data for control brain samples

Primary variable	Variables with statistically significant correlation($p \leq 0.05$) (+/- = Positively/Negatively correlated r, sample size, p)		
Mg	Mn (+0.65, 22, 0.001)	Zn (+0.62, 21, 0.0028)	
Al	---		
Ti	Cl (-0.93, 5, 0.023)	Se (0.90, 6, 0.016)	
Cl	Ti (-0.93, 5, 0.023) Zn (0.52, 20, 0.018)	Br (0.49, 19, 0.035) Na (0.85, 19, 0.0000)	Fe (0.49, 20, 0.028)
K	Mn (0.63, 23, 0.0012)	Zn (0.57, 24, 0.0038)	
Mn	Mg (0.65, 22, 0.0011)	K (0.63, 23, 0.0012)	Cr (0.61, 22, 0.0026)
Sb	---		
Br	Cl (0.49, 19, 0.035) Rb (0.66, 23, 0.0007)	Na (0.65, 23, 0.0007) Zn (0.50, 23, 0.015)	Fe (0.61, 23, 0.0018)
Cd	Cr (-0.77, 7, 0.045)		
Na	Cl (0.85, 19, 0.0000) Br (0.65, 23, 0.0007)	Fe (0.69, 23, 0.0003)	Zn (0.52, 23, 0.012)
Cs	Eu (0.83, 13, 0.0004)		
Cr	Mn (0.61, 22, 0.0026)	Cd (-0.77, 7, 0.045)	
Fe	Cl (0.49, 20, 0.028) Na (0.69, 23, 0.0003)	K (0.39, 24, 0.059) Rb (0.49, 24, 0.015)	Br (0.61, 23, 0.0018) Zn (0.67, 24, 0.0004)
Rb	Br (0.66, 23, 0.0007)	Fe (0.49, 24, 0.015)	Zn (0.63, 24, 0.0011)
Se	Ti (0.90, 6, 0.016)	Zn (0.52, 24, 0.01)	
Zn	Mg (0.62, 21, 0.0028) Br (0.50, 23, 0.0015) Rb (0.63, 24, 0.0011)	Cl (0.52, 20, 0.018) Na (0.52, 23, 0.012) Se (0.52, 24, 0.010)	K (0.57, 24, 0.0038) Fe (0.67, 24, 0.0004)

Chapter 6 Investigation of Principal Component Factor Analysis for Biological Systems

Factor analysis is a statistical procedure used to identify variables in a data set that fluctuate or vary together and separates these elements into individual groups or factors. Examination of the factor loading coefficients for each variable on hypothetical common factors reveals how seemingly unrelated variables relate to one another. In environmental studies, the groups or factors extracted by factor analysis (FA) ideally represent individual sources or source regions. The use of factor analysis in source identification and air quality data analysis has been given by Hopke (1992).

In this study, the principal component factor analysis (PCFA) was performed to identify an element or elements that might correlated differently between the ALS and the control samples of the human brain. In order to appreciate the strengths and limitations of factor analysis, it is important to understand the distinctions between common and unique factors, orthogonal and oblique factors, and variance and covariance. In addition, the concepts of communality, factor loading, factor score and different methods of factor analysis such as factor extraction, factor rotation, and missing value filling should be examined.

A detailed description of the calculations for all values used in Statgraphic 6.0 are given by Johnson and Wichern (1982).

6.1 Factor Patterns: Common, Unique, Orthogonal, and Oblique Factors

Factor analysis assumes that a collection of observed variables can each be expressed as linear combinations of common and unique factors. Common factors appear in the linear combinations of two or more observed variables. Any factor that influences only one observed variable is a unique factor. Common factor refers to the factor that influence two or more observed variables. There are also orthogonal and oblique factors. Unlike the orthogonal factors which are independent each other, oblique factors influence each other.

In addition to assuming the existence of linear relationships between observed variables and hypothetical common and unique factors, factor analysis selects the simpler one between two given factor pattern that describes a collection of observed variables equally well. This postulate is widely accepted and utilized although it cannot be rigorously proven.

Regardless of the complexity of the factor pattern, it is important to note that common factors do not necessarily cause the observed variables. They simply express how the observed variables relate to one to another. Common factors may either be the cause of the samples matrix or the consequence of observed variables.

6.2 Variance and Covariance

In order to determine the factor pattern that describes relationships in a collection of data, factor analysis employs numerous statistical manipulations.

Variance(s) of some quantity X is the average of the square of the deviation of X . This quantity represents how much the variable differs from the average value. Covariance, however, expresses the extent to which one variable varies with another.

Communality is another important concept that is used in statistical analysis. The communality expresses the percent of an observed variable account for by all common factors in a particular path diagram. The communality for any n th variables is the sum of the squares of the factor loading for all common factors. Since the square of an individual correlation coefficient equals the percent of an observed variable attributed to a particular factor, communality represents how much percentage of an element is explained by the factor. In a computer program, estimated communality of 1 represents 100 percent of the variable is explained by the factors extracted.

6.3 Factor Extraction

In factor analysis, the extraction of the common factors determination of the correlation coefficients linking the common factors to each observed elements is very important. In order to accomplish these tasks, factor analysis makes extensive use of both equation and correlation matrices. There are several ways to extract factors depending on the methods in minimizing the resulting residual matrix. Among the possible options are the principal factoring methods and the maximum likelihood procedure. Each of these approaches uses the properties of eigenvectors.

The principle factor method takes advantage of the properties of diagonalized matrices of the form that all components in the matrix are zero except for the diagonal component. The maximum likelihood procedure utilizes the eigenvectors to determine the matrix of common factor loading. Eigenvectors by definition, must be orthogonal to each other. Hence, the eigenvectors of the correlation matrix, multiplied by the square root of the associated eigenvalues yields the matrix of common factor loading. Moreover, eigenvalues correspond to the amount of variance accounted for by each common factor.

Each method of factor extraction extracts enough common factors to account for almost all of the total variance. This usually results in as many common factors as

observed variables, leading to a messy and virtually useless factor model. Prior to reaching this point, however, there are several indications that a sufficient number of common factors have been extracted. Among these indications are the eigenvalue criterion, the Scree test, and the proportion criterion

The eigenvalue criterion stipulates that a common factor is included in a factor model provided the associated eigenvalue is greater than one. This requirement is justified by the observation that the eigenvalue for any common factor indicates the portion of total variance attributed to that factor. Consequently, any factor associated with an eigenvalue less than one, usually accounts for an insignificant portion of the total variance and is omitted from the factor model.

The Scree test also relies upon the eigenvalues identified for each common factor. The eigenvalue for each factor is plotted on a graph. The slope of the resulting line is a measure of the variance extracted per factor. As the slope nears zero and approaches a constant, the same factor is being re-extracted. Therefore, factor extraction is terminated as the slope approaches a constant.

The proportion criterion establishes a requirement for the proportion of the total variance that must be accounted for by the common factors. The proportion of the total variance not accounted for by a given number of common factors, p , is equal to the sum of the diagonal elements in the residual matrix divided by the number of observed variables. With the proportion criterion, common factors are extracted until the value of p drops below some desired value.

When the above criteria conflicts with the number of factors that should be retained, the postulate of parsimony is invoked. Accordingly, the lowest number of common factors that satisfies any of the above stipulations are included in the factor pattern.

6.4 Principal Component Factor Analysis

Principal component factor analysis attempts to simplify the description of a data set by determining a minimum set of basis vectors that span the data space to be interpreted. In this analysis, a standardized variable is used instead of the raw data, so that all variables are treated on an equivalent basis.

In most statistical analyses, there are built in assumptions that the user may or may not desire to use. The most common assumption has to do with deciding on the number of factors to be used. Many routines consider only the eigenvectors having eigenvalues greater than one as significant factors, unless the user explicitly sets other parameters, such as total retained variance, to override the default on the eigenvalues. Although the eigenvalue greater than one criterion sets a lower bound on the number of factors, it does not provide a simultaneous upper limit. In this analysis, Scree test were performed in addition to the eigenvalue greater than one criterion to determine the number of factors to be extracted.

After determine the data matrix, communality values, and number of retained factors, three different rotations: Varimax, Quartimax, and Equamax, were performed. The Varimax rotated loading, which have eigenvalues higher than 0.3, are presented in Table 6-1. The number factors were extracted by the Scree test. The calculated estimated commonalties for each variable using the squared multiple correlation between that variable and all other variables are also given in Table 6-1. Before running the procedure, it must be decided whether the diagonal elements of the correlation matrix will be replaced with these estimated commonalties or not. The diagonal elements were not replaced with correlation or covariance matrix in this study. Missing values in the data set was left as a blank.

6.4.1 Investigation of Factor Rotation

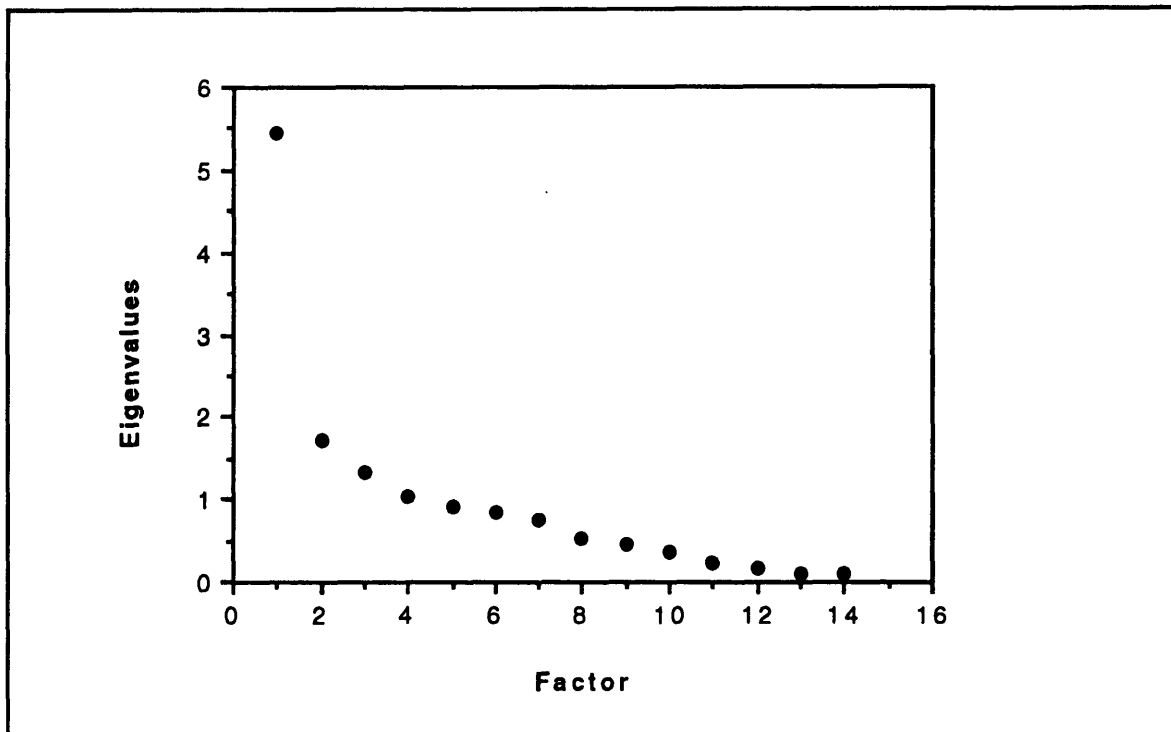
Once the appropriate number of factors have been extracted, the resulting raw factor pattern is transferred via rotation. Rotations simplify the interpretation of the hypothetical factors by reducing the number of common factors with substantial factor loading for each observed variables. Three different rotations are usually used those are Varimax, Quartimax, and Equamax.

The Quartimax rotation simplifies the factorial complexity of each variable. The rotation, although similar to, simplifies the factor pattern by minimizing the number variables dependent on each factor. The rotation establishes coefficients for variables and factors so that the simplicity of the variables and the simplicity of the factors are equally important in determining the simplicity of the over all factor pattern. Although any of these rotations may be used to facilitate factor interpretation, it is difficult to determine which one is better for a analysis. Results of these,, rotated factor analysis are given in Tables 6.1, 6.2, and 6.3. Five factors were extracted by Scree test and eigenvalue criterion for each rotation (Figure 6.1).

The elements having a positive correlation with Br were listed in Factor 1. The elements in Factor 2, Mg, K, Mn, Cr, and Zn, had a positive correlation with Mn. In Factor 3, the factor loading of chlorine was dominant and relatively high loading of Na and Cs in the and Equamax rotations. There was a negatively high value factor loading on Al with a positively high value of Sb in Quartimax rotation. The elemental relation in Factor 3 of the Quartimax rotation was observed in Factor 4 of the and Equamax rotations. Selenium and Zn both had positive factor scores, and Al and Sb had negative factor scores in Factor 5 of the and Equamax rotations. The rotation showed Al, Br, Cs, and Rbin Factor 5.

The Equamax rotations grouped in the same elements in each factor, indicating good agreement in two different rotation methods. Although there were small differences in the extracted factors and the order of the factors was changed in the rotation, the grouped elements were essentially the same as the others. The rotation was chosen for the rest of the analyses since there has been good agreement among different factor rotation methods.

Figure 6.1 Scree test for the extraction of factors



6.4.2 Estimation of Missing Values

It was important to determine what should be done regarding the unknown values. In order to avoid bias in the data set, blank values were replaced by the product of the appropriated detection limits and random numbers. The detection limits represent that the concentrations of a element is smaller than the detection limits but not a exact value. The random number were generated by using a computer program (Excel 5.0).

Table 6.1 Varimax rotated factor loading after PCFA of the data set including missing values in control brain samples.

Element	Loading					Communality
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	
Mg	---	0.89	---	---	---	0.90
Al	---	---	---	- 0.89	-0.33	0.90
Cl	0.34	---	0.86	---	---	0.90
K	---	0.85	---	---	---	0.87
Mn	----	0.87	---	---	---	0.82
Sb	0.35	---	---	0.72	-0.4	0.83
Br	0.90	---	---	---	---	0.88
Na	0.51	---	0.75	---	---	0.84
Cs	---	---	0.74	---	---	0.61
Cr	---	0.63	---	0.50	---	0.81
Fe	0.68	---	0.42	-0.34	---	0.85
Rb	0.85	---	---	---	----	0.81
Se	---	---	---	---	0.97	0.99
Zn	0.65	0.50	0.33	---	0.30	0.91

Table 6.2 Quartimax rotated factor loading after PCFA of the data set including missing values in control brain samples.

Element	Loading					Communality
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	
Mg	---	0.88	---	---	---	0.91
Al	---	---	-0.86	-0.37	0.41	0.90
Cl	0.73	---	---	-0.33	---	0.90
K	---	0.84	---	0.30	---	0.87
Mn	---	0.86	---	---	---	0.82
Sb	---	---	0.74	-0.31	---	0.83
Br	0.84	---	---	---	-0.35	0.88
Na	0.83	---	---	---	---	0.84
Cs	---	---	---	---	0.69	0.61
Cr	---	0.64	0.53	---	---	0.81
Fe	0.84	---	---	---	---	0.85
Rb	0.66	---	---	0.40	-0.46	0.81
Se	---	---	---	0.95	--	1.00
Zn	0.75	0.46	---	---	---	0.91

Table 6.3. Equamax rotated factor loading after PCFA of the data set with missing values in control brain samples.

Element	Loading					Communality.
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	
Mg	---	0.89	---	---	---	0.91
Al	---	---	---	-0.89	-0.33	0.90
Cl	---	---	0.88	---	---	0.90
K	---	0.84	---	---	0.33	0.87
Mn	---	0.88	---	---	---	0.82
Sb	0.38	---	---	0.72	-0.37	0.83
Br	0.89	---	---	---	---	0.88
Na	0.46	---	0.78	---	---	0.84
Cs	---	---	0.73	---	---	0.61
Cr	-0.38	0.62	---	0.51	---	0.81
Fe	0.62	---	0.47	-0.35	0.33	0.85
Rb	0.83	---	---	---	---	0.81
Se	---	---	---	---	0.99	1.00
Zn	0.60	0.49	0.37	---	0.38	0.91

6.4.3 Principal Component Factor Analysis of Control and ALS brain data sets

Tables 6-4 and 6-5 give the rotated factor loading after PCFA of the data set without missing values in control and ALS brain tissues. As expected, extracted factors without missing values were different from those of with values of missing. When factor analyses

Table 6.4 Varimax rotated factor loading after PCFA of the data set without missing values in control brain.

Element	Loading					Communality
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	
Mg	0.45	0.76	---	---		0.82
Al	0.44	---	---	-0.34	-0.40	0.57
Cl	0.88	---	---	---	---	0.91
K	---	0.73	0.41	---	---	0.77
Mn	---	---	0.95	---	---	0.92
Sb	---	---	---	0.83	---	0.79
Br	0.72	---	---	0.41	---	0.83
Na	0.93	---	---	---	---	0.93
Cs	---	---	---	---	0.85	0.79
Cr	---	0.74	---	---	---	0.68
Fe	0.78	---	0.47	---	---	0.83
Rb	---	---	0.60	0.60	-0.34	0.93
Se	---	---	0.93	---	---	0.90
Zn	0.54	0.38	0.58	---	---	0.85

Table 6.5 Varimax rotated factor loading after PCFA of the data set without missing values in ALS brain.

Element	Loading					Communality
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	
Mg	0.84	---	---	---	---	0.79
Al	---	---	---	0.75	---	0.67
Cl	---	0.84	---	0.30	---	0.83
K	0.79	---	---	---	---	0.67
Mn	0.89	---	---	---	---	0.83
Sb	---	---	---	0.68	0.40	0.71
Br	---	---	0.68	---	---	0.55
Na	---	0.87	---	---	---	0.92
Cs	---	---	---	0.74	---	0.66
Cr	---	---	---	---	0.85	0.73
Fe	---	---	0.61	---	---	0.44
Rb	---	0.73	---	---	---	0.55
Se	---	---	0.75	---	---	0.68
Zn	---	---	0.66	---	---	0.64

are performed with missing values, unknown concentrations are recognized as zero values which is not true. Therefore, results obtained without missing values are more appropriate for the analysis. Five factors were extracted by the Scree test and eigenvalue criteria.

The results of the PCFA of control samples without missing values are given in Table 6.4. High factor loadings of Cl, Br, Na and Fe were observed in Factor 1. When the elements in Factor 1 is compared with the correlation analysis results, it can be concluded that Factor 1 represents elements having correlation with Na and Cl.

Equally weighted Mg, K, and Cr were extracted in Factor 2. These elements are strongly correlated with Mn in the brain. There might be a certain association among those elements with Mn, although the specific role of Mn in the brain is unknown. The involvement of Zn in Factor 2 is ignored since the factor loading was low compared with other elements in that factor. Factor 3 contains K, Mn, Fe, Rb, Se, and Zn. The relations of the elements in Factor 3 is not clear.

Aluminum and Fe having negative factor loading, and Sb, Br, and Rb having positive values, were observed in Factor 4. This factor suggests that the function of Al in the brain may be negatively correlated with the rest of the elements in Factor 4. A high factor loading of cesium was obtained in Factor 5. This factor is ignored since the data of cesium was associated with high uncertainty in determining its concentration. The implications of factors obtained from PCFA of control brain tissues are not clear. However, they may represent a certain role of elements.

The results of PCFA for the ALS brain samples are quite different as compared with that of control samples. Factor 1 involved Mg, K, and Mn which had a positive correlation in concentration levels of ALS brain samples. In the control sample, Na and Cl were grouped with Fe and Br.

As shown in Factor 2, Na and Cl are associated with Rb. This result is quite interesting because the concentrations of Rb in ALS samples taken from brain were lower than that in the control samples whereas the concentration of Na and Cl were similar in both. If we can explain the neurotoxic effect of Rb in brain tissues in relation with the concentration of Na or Cl, the role of Factor 2 on the formation of neurofibrill could be

explained. Bromine, Fe, Se, and Zn were grouped in Factor 3. Factor 3 may indicate elements associated with selenium function in the body. One of the functions of Se is the metabolism of toxic and essential heavy metals in the body. It is known that Se deficiency causes many diseases such as muscle and heart disease. The effects of Factor 3 can be explained if we can show the relationships among the elements found in Factor 3. Factor 4 involves Al, Cl, Sb, and Cs. A high factor loading of Cr was observed in Factor 5 of the ALS data. The abnormal distribution of the Cr level in the motor cortex of ALS were observed from SKC. By relating this result with PCFA, Factor 5 can be explained as the element that shows wide distribution in a degenerated brain.

6.4.4 PCFA of mixed samples and Factor Scores.

Factor scores are very helpful in interpreting and understanding PCFA results. They also can be helpful in finding errors that may exist in data sets since one distinct high value represents a data noise attributed to the analytical methods used in the elemental determination. In addition, factor scores are essential in understanding whether the correlations among the elements in a factor represent correlations observed for the entire data set or represent unusual concentrations observed in one or two samples. With factor scores, the origin of a sample can be found if there is no big error in analytical methods.

In order to see whether we can separate different tissues from different origins or different sources contributing to the tissues, PCFA was performed with all mixed seventy-two samples that were obtained from different region of the body. After performing the rotation to obtain the factor loading, the factor scores were calculated from the standardized data and the rotated factor loading by a least-squared method (Statgraphics plus 6.0 Manugistics). By Scree test and eigenvalue greater than one criterion, five factors were extracted. Elements with many missing values were eliminated from the data set which were Ti, V, Cd, and Eu.

Table 6.6 Varimax rotated factor loading after PCFA of the data set without missing values in 72 samples.

Element	Loading					Communality
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	
Mg	0.67	---	---	---	---	0.80
Al	0.42	---	0.64	---	---	0.70
Cl	0.65	-0.35	---	0.41	---	0.82
K	0.88	---	---	---	---	0.79
Mn	0.64	0.63	---	---	---	0.80
Sb	0.60	---	---	---	---	0.95
Br	---	---	---	---	0.96	0.52
Na	0.83	---	---	---	---	0.94
Cs	---	---	0.90	---	---	0.77
Cr	---	0.82	---	---	---	0.72
Fe	0.59	-0.44	---	0.33	0.33	0.74
Rb	---	---	---	0.87	---	0.66
Se	0.78	---	---	---	---	0.69
Zn	0.92	---	---	---	---	0.81

Figures 6.2, 6.3, 6.4, 6.5, and 6.7 show the plots of the factor scores obtained from PCFA of seventy-two samples. These samples include motor cortex tissues, cerebellum tissues, and spinal cords taken from both degenerated and healthy sites.. There

were different trends in factor scores between the brain and spinal cord samples. From this observation, variables in Factor 1 can be regarded as elements that are related to brain tissue but not in spinal cords. Factor scores in Factor 2 are high in spinal cords and low in brain samples indicating elements related in spinal cords (Figure 6.3). Due to the high variation in the factor scores of Factors 3,4, and 5, among the various samples, the implication of those factors are not clear.

Factor scores also used to find any possible errors that may be exist in data set. Samples showing factor scores greater than 3 or smaller than -3 are regarded as error and should be extracted from analyses. In this study, the factor scores of all samples were ranged from 3 to -3, indicating that the concentrations of elements in each sample obtained from instrumental neutron activation analysis (INAA) does not include significant errors. In order words, the analytical methods used in the elemental determination is fairly accurate.

Figure 6.2 Factor 1 scores from PCFA of 72 samples

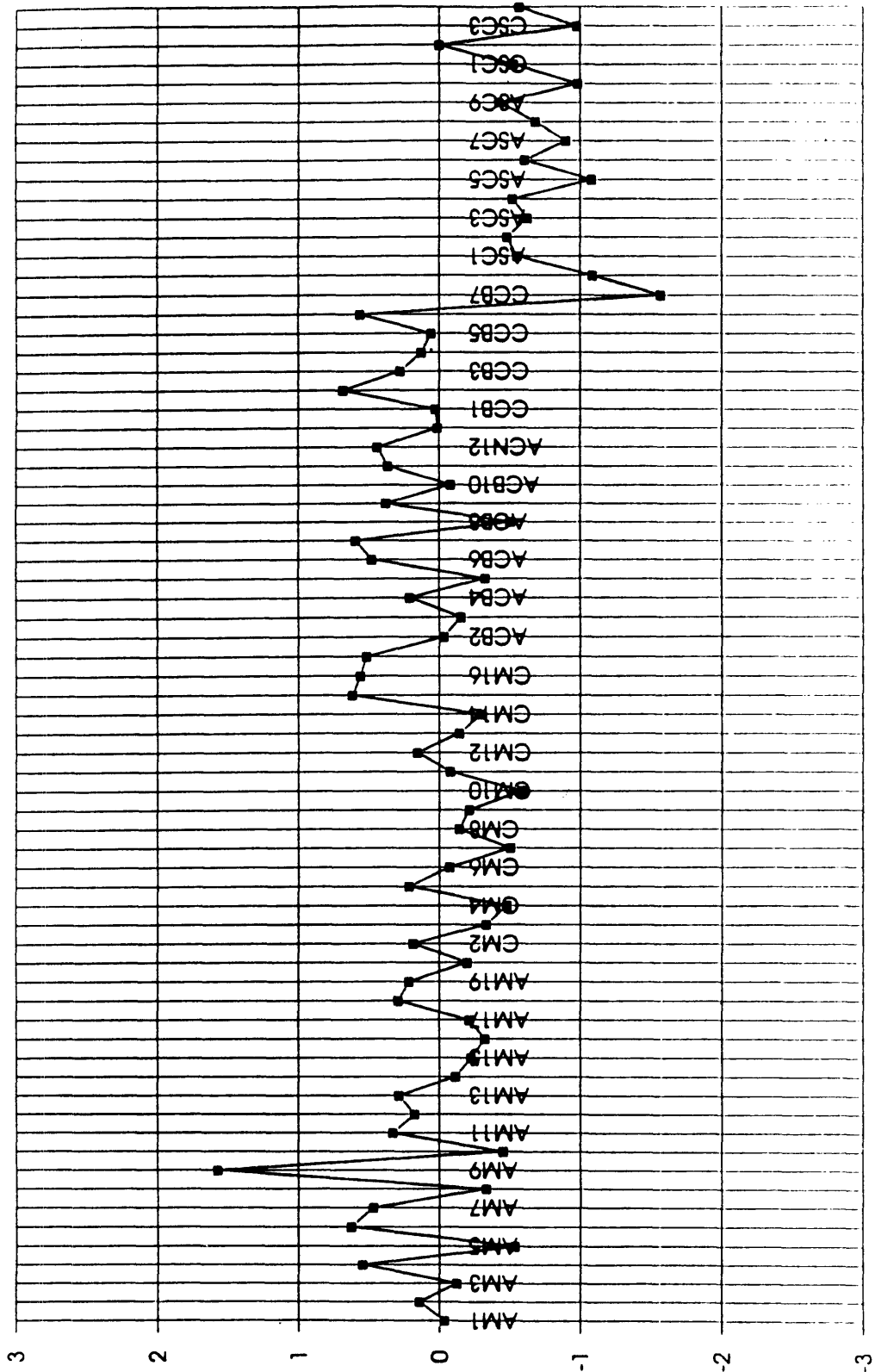


Figure 6.3 Factor 2 scores from PCFA of 72 samples

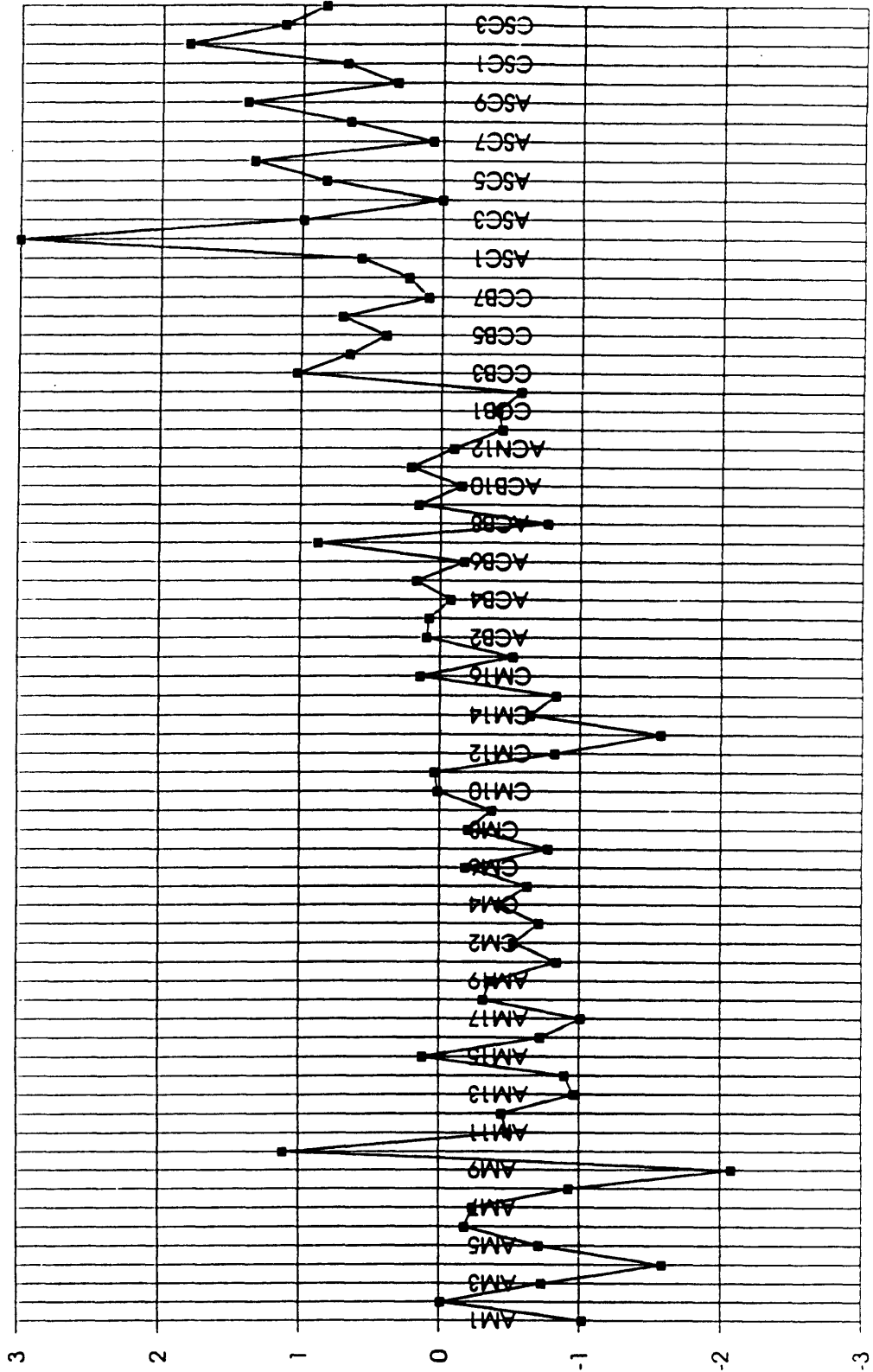


Figure 6.4 Factor 3 scores from PCFA of 72 samples

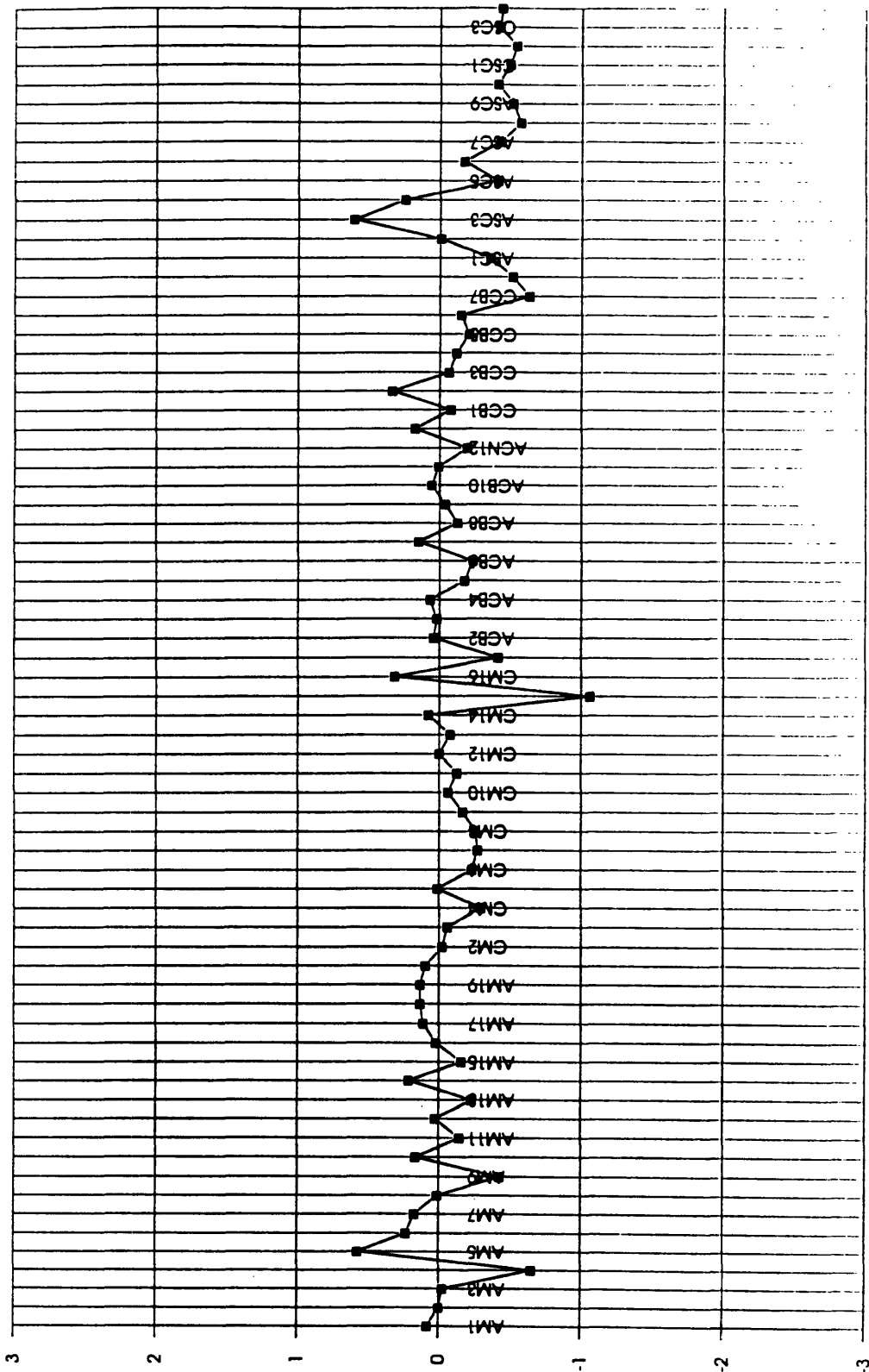


Figure 6.5 Factor 4 scores from PCFA of 72 samples

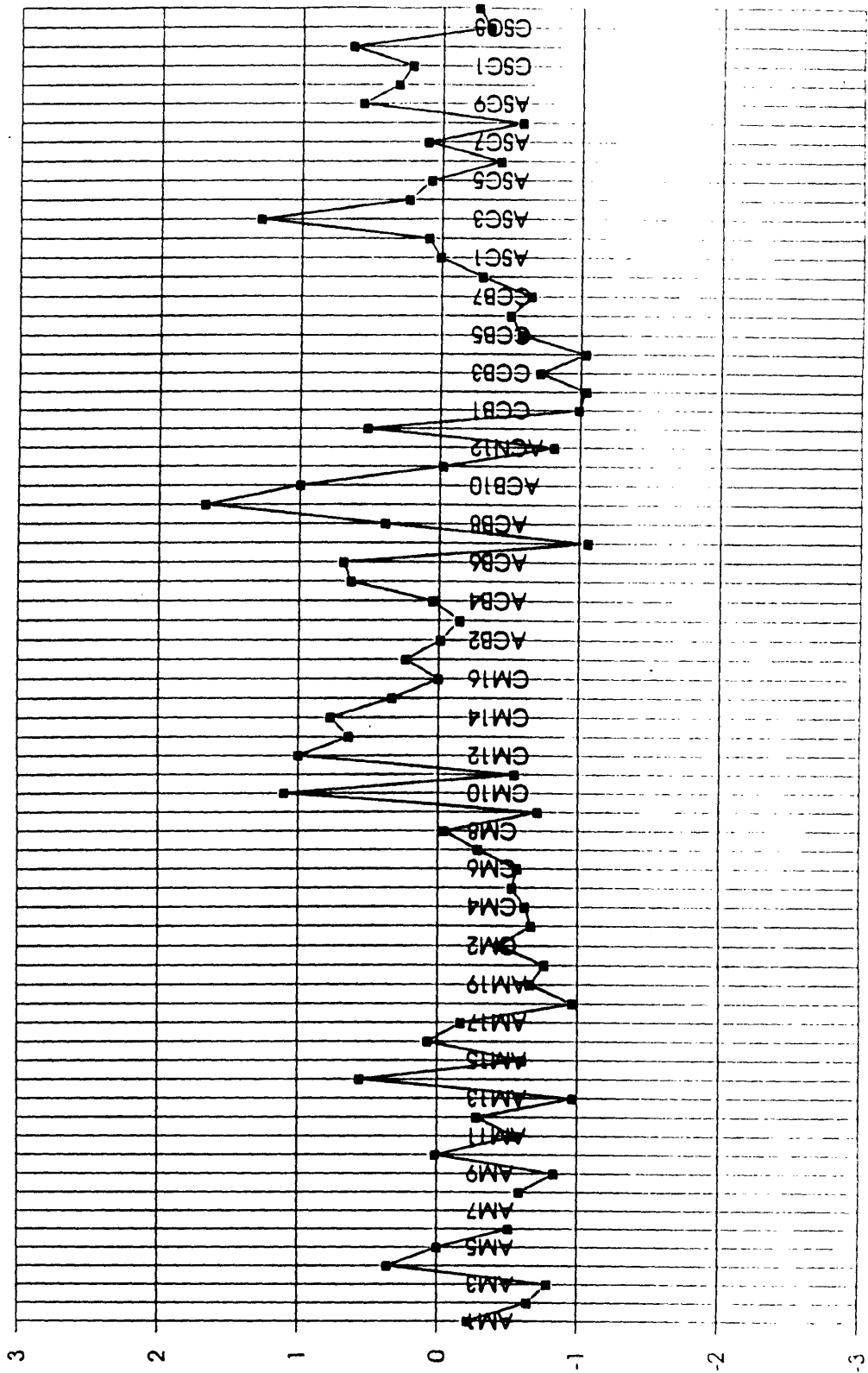
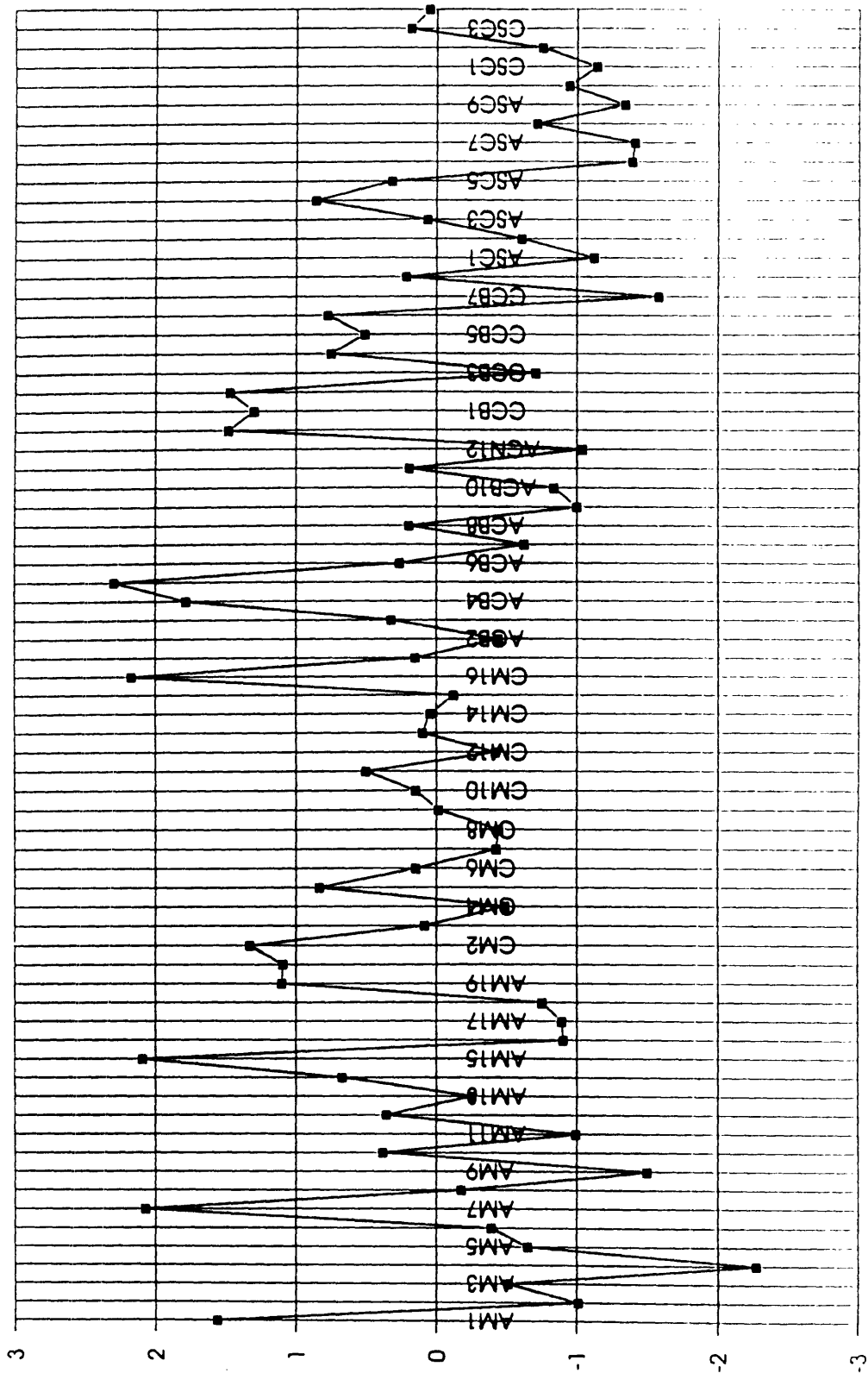


Figure 6.6 Factor 5 scores from PCFA of 72 samples



Chapter 7 Conclusions

A neutron activation analysis has been used to determine the concentrations of trace and minor elements in biological systems. Using this technique, concentrations of eighteen elements were obtained for a total of seventy two biological samples. Despite its advantage over the other nuclear technique to detect very low levels of elements in samples, missing values were also observed in certain elements. This may be the result of high background noise, short half life, and other factors.

Summary statistics have been applied to the data sets to calculate the averages. They have been also used to calculate the SKC so that the shape of the distribution could be examined. The important result given by the summary statistics is the differences in average values between ALS and the control samples. High levels of Al, Sb, Cr, Eu, and Se and low levels of Br, Cd, and Rb have been observed in the ALS samples taken from motor cortex. Different results were drawn from the cerebellum data, which showed elevated levels of Al, Mn, Sb, Br, Cs, and Eu and low levels of Cr, Rb and Se in ALS. The comparison of average values for spinal cord were not appropriate because of relatively small sample number. Even so, it is included because it still provides the range of the elemental content in spinal cord.

The correlation analysis study was performed to obtain the elemental relationships between the samples. Different elemental correlations were observed between ALS samples and the control samples. In ALS samples, the elemental correlations were much

simpler than in those of the control. Because of the limited knowledge between the relationships of trace and minor elements in the human body, it is not clear whether the elemental correlations in tissue provide an information in understanding the characteristics of samples. And if so, what is the possible underlying mechanism.

In order to examine the possibility of finding the elements that affect the causation of ALS, principal component factor analysis (PCFA) were applied to the data sets. Significantly different factor patterns were observed between the ALS and the control samples. Some of the factors obtained from the control brain sample data were explained in terms of correlations and functions of elements in body. In order to better understand the impact of factors or to explain what the each factor stands for, more information about the interactions of elements in body and the roles of these elements are necessary.

Finishing the PCFA, Rb was found to be one of the most interesting element. The significant differences in the average concentration values were observed between in the ALS and control samples. In addition, the Rb, Na, and Cl were grouped in a same factor for ALS samples that did not shown in the control samples. Although it was not possible either to explain the Factor or to relate the Factor to the causation of ALS disease at this point, it might be important to focus our study on the association of Rb with other elements such as Na and Cl.

The factor scores were plotted to examine whether we can separate samples having same origin from mixed samples. The factor scores of the brain samples were positive for a factor including Mg, Al, Cl, K, Mn, Sb, Na, Fe, Se, and Zn. And factor scores of spinal cord samples were positive for a factor including Cl, Mn, Cr, and Fe. The relationships of the elements in a factor are not clear. However, these results provide the feasibility of PCFA in separating samples of same origin from mixed origin of samples and in finding errors that may exist in data sets. In this study, the Factor 1 and Factor 2 are explained in

terms of the origin of samples that are brain tissue and spinal cord, respectively. In order to explain the rest of factors, more sample information, such as age or sex is needed.

The most important significance of this study is that it provides a library of concentration ranges of most of the elements in motor cortex, cerebellum, and spinal cords. No other studies have analyzed as much samples as have been studied here. Also, no other studies have examine the applicability of factor analysis on biological data sets. This study also indicates that it is important to analyze samples separately because the elemental contents vary from one region to another.

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APPENDIX A INAA Results

	Brains (ug/gr)		ALS1 +/-		ALS2 +/-		ALS3 +/-		ALS4 +/-		ALS5 +/-		ALS6 +/-		ALS7 +/-		ALS8 +/-	
	AM1	AM2	AM3	AM4	AM5	AM6	AM7	AM8	AM9	AM10	AM11	AM12	AM13	AM14	AM15	AM16	AM17	AM18
Mg	642	107	143	783	143	700	140	563	94	630	114	965	192	976	196	540	110	
Al	4.2	2.9	1.3	4	1.3	5.4	dl	10	2.1	21	3.4	3.7	2.2	22	4.3	5.8	dl	
Ti	9.9	7.3		25	dl	32	dl	16	dl	34	dl	38	dl	462	44	33	dl	
V	0.19	dl		0.13	dl	0.2	dl	0.13	dl	0.21	dl	0.22	dl	0.34	dl	0.23	dl	
Cl	7150	4010	2840	4790	2840	5680	3630	5300	2970	6880	3650	8210	4870	12870	14380	6410	4080	
K	10490	1460	2550	11700	2550	8590	2450	8940	1140	8550	1340	11900	2670	12370	2850	8880	2590	
K(L)	10040	1260	1490	13040	1490	11660	1300	10480	1000	10120	2700	13500	1220	13500	1630	8888	1270	
K (ave)	10265			12370		10125		9710		9335		12700		12935		8884		
Mn	0.77	0.12	0.16	1.2	0.16	0.67	0.17	1.1	0.11	1.3	0.19	1.6	0.31	1.7	0.3	0.64	0.18	
Sb(L1)	0.074	dl		0.096	dl	0.059	0.03	0.062	dl	0.095	dl	0.15	dl	0.191	dl	0.091	dl	
Sb(L2)	0.28	dl		0.14	dl	0.075	dl	0.572	0.189	0.327	0.14	0.0547	0.0563	0.216	dl	0.047	dl	
Br	2.8	0.22	62	231	62	5.8	0.17	1.3	0.4	20	5.5	5.5	0.66	5.08	1.48	8.2	2.3	
Cd	0.32	dl		0.39	dl	0.51	dl	0.26	dl	0.92	dl	0.16	0.29	0.873	dl	0.19	0.14	
Na(L)	6930	449	352	5426	352	6280	408	5720	370	7716	502	7483	487	13190	860	6470	420	
Na(SH)	6300	420	360	5100	360	5200	380	4400	290	7300	490	7800	540	11100	770	5700	420	
Na(ave)	6615			5263		5740		5060		7508		7641.5		12145		6085		
Cs	0.11	0.053		0.084	dl	0.033	dl	0.11	dl	0.78	dl	0.063	0.024	0.367	0.0637	0.053	0.013	
Cr	0.66	0.44	0.4	2.68	0.4	0.58	0.17	1.6	0.58	1.4	0.43	2.15	0.24	0.98	0.46	0.447	0.174	
Eu	0.046	dl		0.025	dl	0.0064	dl	0.034	dl	0.018	dl	0.017	0.0077	0.108	0.0196	0.0096	0.003	
Fe	467	55	29	494	29	274	18	379	37	276	27	358	16.9	403	42	288	14.7	
Rb	17	3.1	1.4	4.3	1.4	5.8	1.4	3.4	2.4	6.102	2.07	2.78	0.668	16.5	1.92	7.33	0.403	
Se	1.2	39	0.49	1.5	0.49	1.13	0.203	0.45	0.5	0.89	0.5	1.24	0.372	0.884	0.37	0.911	0.228	
Zn	40	5.3	1.1	49	1.1	26	5.1	36	1.3	32	6.4	41	5.3	50.8	6.11	29	3.8	

	ALS9 AM9	ALS10 AM10	ALS11 AM11	ALS12 AM12	ALS14 AM13	ALS15 AM14	ALS16 AM15	+	+	+	+	+	+	+
Mg	558	105	607	103	833	160	524	102	612	115	607	108	618	103
Al	3.3	1.8	5.6	1.5	4.9	3	5.3 dl		5.5 dl		3.9 dl		3.9	1.6
Ti	22 dl		9.9	7.4	34 dl		7.8	7.9	35 dl		3.5	7.5	16 dl	
V	0.18 dl		0.075	0.035	0.25	0.079	0.2 dl		0.22 dl		0.14 dl		0.14 dl	
Cl	9810	5500	5680	3010	5040	2990	9830	5510	6820	4330	6950	3680	7250	4060
K	10740	1440	10860	1580	10640	2330	6940	1080	8990	2630	8720	1320	9150	1190
K(L)	16710	2700	8262	1425	13030	2610			18200	890	10600	1590	10940	1060
K (ave)	13725		9561		11835		6940		13595		9660		10045	
Mn	0.83	0.15	1.3	0.13	1.3	0.18	1.7	0.2	0.69	0.24	1.5	0.15	0.77	0.11
Sb(L1)	0.066 dl		0.097 dl		0.11	45	0.15 dl		0.098 dl		0.07 dl		0.025	0.031
Sb(L2)	0.51	0.24	0.052	0.025	0.37		0.086 dl		0.1	0.092	0.076	0.071	0.11 dl	
Br	2.6	0.73	5.7	1.6	1.5	0.51	4.5	1.4	3.4	0.26	5.46	1.51	7.4	2
Cd	0.31 dl		0.64 dl		0.72 dl		1.1 dl		0.81 dl		0.0689	0.147	0.16	0.09
Na(L)	9740	630	6803	442	5230	340	11000	690	10530	179	8346	543	9050	586
Na(SH)	7600	500	6500	440	4900	340	8400	560	6000	440	6900	460	6600	440
Na(ave)	8670		6651.5		5065		9700		8265		7623		7825	
Cs	0.13 dl		0.089	0.023	0.065 dl		0.1	0.03	0.045 dl		0.2	0.0334	0.045	0.018
Cr	2.5	0.57	9.7	0.28	2.48	0.471	2.06	0.206	0.81	0.43	0.456	0.262	4.1	0.9
Eu	0.04 dl		0.0098	0.0036	0.015 dl		0.037	0.011	0.0085 dl		0.04	0.009	0.018 dl	
Fe	510	51	313	15	362	32	380	23	307	21	479	26	275	19.8
Rb	4.6	1.9	8.9	0.94	4.42	3.1	7	0.89	7.7	1.8	10.3	1.1	21	3
Sc	1.4	0.59	0.4	0.14	0.842	0.33	1.06	0.255	1.4	0.26	0.42	0.14	0.97	0.16
Zn	55	7.2	29	3.8	31	6.1	46	6	34	6.6	42	4.9	45	5.9

	ALS17	+/-	B3	+/-	B7	+/-	B27	C1	C2	C3
	AM16	AM17	AM18	AM19	AM18	AM19	CM1	CM2	CM3	
							X12	X13	X14	+/-
Mg	677	122	621	102	927	162	473	710	143	90
Al	0.74	0.55	2.9 dl		2.4 dl		2	1.1	2	5.6 dl
Ti	7.2	5.1	7.2	3.7	16 dl		13	7.3	31 dl	15 dl
V	0.098 dl		0.11 dl		0.095 dl		0.16 dl	0.049 dl		0.15 dl
Cl	5090	3020	5490	2910	4590	2720	5680	3010	8930	5040
K	8710	1890	10290	1460	13800	2950	9930	1420	8270	7440
K(L)							15120	1740	12630	12800
K (ave)	8710		10290		13800		12525		10450	10120
Mn	0.95	0.13	0.65	0.063	1.7	0.12	0.87	0.088	1.4	0.2
Sb(L1)	0.041	0.022	0.0093 dl		0.0091 dl		0.026	0.022	0.03	0.017
Sb(L2)	0.0905	0.0326					0.039 dl		0.15 dl	0.041 dl
Br	1.3	0.14	1.5	0.51	0.98	0.33	12.2	3.3	4.6	1.3
Cd	0.158	0.132	0.022 dl		0.029 dl		0.26	0.16	0.23 dl	0.35
Na(L)	6473	420	6657	742	4498	563	7460	484	8740	566
Na(SH)	4900	340	5600	380	4100	290	5500	370	7600	4400
Na(ave)	5686.5		6128.5		4299		6480		8170	5676.5
Cs	0.166	0.0221	0.13	0.022	0.045	0.012	0.05	0.021	0.092 dl	0.048
Cr	0.715	0.114	0.28	0.081	0.11	0.11	0.53	0.18	1.3	0.33
Eu	0.0178	0.0393	0.009	0.007	0.016	0.004	0.0047 dl		0.029 dl	0.0058
Fe	290	14.3	316	17	336	16	365	19.2	384	286
Rb	1.11	0.29	1.2 dl		1.5	0.44	16.14	3.43	13.5	1.6
Se	0.581	0.122	0.43	0.14	0.77	0.18	0.85	0.29	0.64	0.21
Zn	35.5	4.21	45	5.8	44	5.5	40	7.9	54	39

	C12	C13	C14	C15	B11	B40	B1	+/-
	X25 +/-	H1 +/-	H2 +/-	H03 +/-	CM15 +/-	CM16 +/-	ACBI	
	CM11	CM12	CM13	CM14	CM15	CM16		
	No SH1							
Mg	695	530	469	520	629	1120	670	150
Al		29 dl	4.8 dl	19	1.7	4.7 dl	9.4 dl	
Ti		75 dl	5.1	20 dl	5.1	32 dl	55 dl	
V		0.62 dl	0.17 dl	0.13 dl	0.022	0.0056	0.36 dl	
Cl	9140	7890	9080	6520	7200	11030	10050	6380
K	10600	8800	6280	8900	11430	10940	15500	4490
K(L)	10880	14200	10700	9140				
K (ave)	10740	11500	8490	9020	11430	10940	15500	
Min	1.6	0.64	0.56	0.97	1.6	1.9	1.9	0.32
Sb(L1)	0.053 dl	0.12 dl	0.141	0.0602	0.63	0.021 dl	0.63 dl	
Sb(L2)	0.15 dl	0.069 dl	0.22	0.092 dl				
Br	4.6	7.3	5.19	7.5	8.2	13	8.1	3.7
Cd	0.025 dl	0.83 dl	0.492 dl	0.19 dl	0.23 dl	0.06 dl	0.19 dl	
Na(L)	7980	9350	10840	7800	8297	10990	7064	1031
Na(SH)		7600	7000	6800	6200	9900	8400	620
Na(ave)	7980	8475	8920	7300	7248.5	10445	7732	
Cs	0.081 dl	0.083	0.201	0.059 dl	0.14	0.12	0.12	0.052
Cr	3.3	0.712	0.677	0.89	2.3	1.4	1.9	0.66
Eu	0.029	0.012	0.048	0.015 dl	0.017	0.029	0.058	0.022
Fe	297	293	404	360	213	375	287	42
Rb	8.2	8.1	8.34	10	17	16	11	1.9
Se	0.51	1.1	0.678	0.3	0.49	0.89	0.83	0.42
Zn	29	39	34.4	32	53	55	36	5.5

	B8	+/-	B12	+/-	B14	+/-	B17	+/-	B20	+/-	B23	+/-	B26	+/-
	ACB2		ACB3		ACB4		ACB5		ACB6		ACB7		ACB8	
Mg	990	170	960	177	870	160	819	139	750	170	960	168	595	100
Al	6 dl		5.3	2.3	3.7	1.9	4 dl		17	4.1	1.8	0.86	4.8 dl	
Ti	33 dl		27 dl		38 dl		26 dl		56 dl		17 dl		18 dl	
V	0.054	0.038	0.17 dl		0.26 dl		0.15 dl		0.38 dl		0.094 dl		0.16 dl	
Cl	3340	2120	6650	3950	6840	4350	5980	3170	11650	7400	4550	2700	7640	4280
K	14900	4190	12640	2730	15700	4420	15810	2270	12700	3790	15800	3370	7390	1040
K(L)														
K (ave)	14900		12640		15700		15810		12700		15800		7390	
Mn	1.9	0.18	1.5	0.15	2	0.22	1.7	0.17	1.9	0.38	2.4	0.14	0.79	0.11
Sb(L1)	0.018	0.021	0.01	0.0051	0.032	0.086	0.11 dl		0.028	0.0082	0.014 dl		0.028 dl	
Sb(L2)														
Br	0.16	0.072	11	3.6	2.4	0.82	8.2	3.9	9.9	3.1	1.5	0.52	2.5	1.1
Cd	0.18 dl		0.03 dl		0.043 dl		0.43 dl		0.033 dl		0.049 dl		0.075 dl	
Na(L)	3171	468	4550	478	6354	796	6915	1295	7830	823	4568	510	5924	861
Na(SH)	3900	290	5600	390	6000	440	7100	470	9900	720	4400	300	5900	390
Na(ave)	3535.5		5075		6177		7007.5		8865		4484		5912	
Cs	0.14	0.062	0.077	0.019	0.15	0.041	0.22	0.065	0.058	0.021	0.033	0.012	0.18	0.057
Cr	0.27	0.25	0.33	0.072	1.4	0.22	0.87 dl		0.59	0.1	4.5	0.19	0.52 dl	
Eu	0.019	0.006	0.009	0.003	0.028	0.013	0.058	0.028	0.015	0.005	0.011	0.0023	0.015	0.0089
Fe	280	25	177	10.3	443	27	268	31	218	14	302	15	238	25
Rb	4.2	0.86	9	0.81	17	2	22	2.9	9.9	0.99	2.1	0.4	7.8	1.5
Se	0.51	0.2	0.5	0.11	0.84	0.26	0.55	0.3	0.91	0.23	0.91	0.23	1	0.39
Zn	36	5.4	33	3.8	41	5.2	33	5	39	4.5	54	6.8	30	4.5

	B29	+/-	B30	+/-	B34	+/-	B36	+/-	B39	+/-	B5	+/-	B18	+/-
	ACB9		ACB10		ACB11		ACB12		ACB13		CCB1		CCB2	
Mg	792	139	697	121	870	190	803	137	929	174	702	117	591	96
Al	26	4.5	9.9	dl	9.2	dl	4.7	dl	4.2	2.5	4.2	dl	3.4	dl
Ti	41	dl	27	dl	48	dl	11	11	16	14	17	dl	14	dl
V	0.26	0.068	0.25	dl	0.33	dl	0.17	dl	0.095	0.078	0.15	dl	0.12	dl
Cl	5390	2860	6650	3730	8210	5250	5630	2990	7750	4590	7530	4220	6150	3450
K	18000	2570	15800	1930	13100	3770	17390	2480	13900	3040	11900	1500	11940	1480
K(L)														
K (ave)	18000		15800		13100		17390		13900		11900		11940	
Mn	2.2	0.18	2.1	0.16	2	0.35	2	0.17	2.1	0.22	1.2	0.13	0.85	0.095
Sb(L1)	0.026	dl	0.09	0.024	0.012	0.006	0.025	dl	0.06	dl	0.0062	0.003	0.017	dl
Sb(L2)														
Br	1.4	0.48	3	1.3	10	3.1	1.2	0.46	2.9	1.4	7.4	2.3	5.8	2
Cd	0.059	dl	0.11	dl	0.027	0.011	0.081	dl	0.99	dl	0.019	dl	0.019	0.019
Na(L)	4965	622	3676	535	7400	780	5211	654	4791	699	7395	777	6580	735
Na(SH)	5300	360	5500	370	7300	540	5800	390	6600	460	5800	390	4500	300
Na(ave)	5132.5		4588		7350		5505.5		5695.5		6597.5		5540	
Cs	0.16	0.06	0.27	0.098	0.059	0.014	0.056	0.025	0.18	0.078	0.028	0.013	0.11	0.02
Cr	0.87	0.31	2.4	0.58	1.4	0.092	0.32	0.29	0.99	dl	0.94	0.083	1.9	0.15
Eu	0.061	0.038	0.097	0.033	0.0064	dl	0.054	0.01	0.046	dl	0.012	dl	0.011	0.002
Fe	281	43	307	48	243	11	229	19	495	51	303	15	414	18
Rb	4.4	1.8	4.6	dl	8.3	0.73	1.5	0.62	14	2.4	15	1.3	18	1.7
Se	0.74	0.32	0.47	0.34	0.69	0.14	1.2	0.41	0.48	0.26	0.86	0.16	3.5	0.65
Zn	37	4.9	29	4.8	46	5.2	35	4.3	27	4.2	46	5.3	60	7.7

	B21	+/-	B22	+/-	B25	+/-	B28	+/-	J3	+/-	J7	+/-	J9	+/-
	CCB3		CCB4		CCB5		CCB6		CCB7		CCB8		CCB9	
Mg	742	127	736	131	731	124	960	190	130	25	583	105	888	157
Al	3.3 dl		2	1.1	3.4 dl		8	4.4	2.1	1.2	5.8 dl		15	4.7
Ti	21 dl		16 dl		22 dl		53 dl		3.3	3.9	24 dl		11	10
V	0.13 dl		0.14 dl		0.13 dl		0.42 dl		0.092 dl		0.16 dl		0.26 dl	
Cl	6820	3620	6300	3530	4340	2300	6900	4380	865	480	1599	1073	1308	914
K	13650	1950	15520	1870	16240	2300	15600	4420	3517	385	7995	912	10360	1420
K(L)									2149	213	10240	670		
K(ave)	13650		15520		16240		15600		2833		9117.5		10360	
Mn	2.1	0.16	2	0.14	1.8	0.15	2.7	0.3	0.88	0.056	1.1	0.085	0.88	0.097
Sb(L1)			0.041 dl				0.0068	0.0053	0.0057 dl		0.0028	0.0034		
Sb(L2)	0.017 dl				0.014	0.011								
Br	1.5	0.5	3.8	1.7	1.6	0.57	2.8	0.9	1.1	0.31	2.2	0.57		
Cd	0.062 dl		0.087 dl		0.043	0.033	0.018 dl		0.017 dl		0.027 dl			
Na(L)	7278	913	4525	673	4110	518	6340	666	2900	190	2900	180		
Na(SH)	7100	470	4600	300	4500	300	6200	450	5000	330	3000	200	3900	260
Na(ave)	7189		4562.5		4305		6270		3950		2950		3900	
Cs	0.06	0.018	0.024 dl		0.11	0.025	0.088 dl		0.02	0.013	0.102 dl			
Cr	6.9	0.21	4.8	0.55	2.5	0.17	2.2	0.14	2.5	0.084	0.7	0.16		
Eu	0.02	0.0055	0.075 dl		0.028	0.0068	0.032 dl		0.016 dl		0.039 dl			
Fe	228	13	287	56	228	14	313	24	25	14	83	25		
Rb	0.93	0.43	12	3.5	12	1.2	12	1.5	3.1	0.77	13	2		
Se	0.78	0.22	0.81	0.37	1.4	0.29	0.71	0.16	0.23	0.089	0.43	0.25		
Zn	43	5.4	39	6.3	38	4.7	47	5.5	9.8	1.3	17	2.4		

	J2	+/	J6	+/	J10	+/	J14	+/	J17	+/	J18	+/	J19	+/
	ASC1		ASC2		ASC3		ASC4		ASC5		ASC6		ASC7	
Mg	629	131	462	124	726	151	1076	239	596	110	830	149	392	77
Al	9	dl	4.5	9.7	20	dl	7.3	4.9	6.7	dl	4.9	2.2	6.7	dl
Ti	24	19	13	33	75	dl	23	32	32	dl	13	11	6.8	8.4
V	0.31	dl	0.52	0.25	0.51	dl	0.58	0.024	0.099	0.054	0.068	0.058	0.03	0.059
Cl	3343	2251	3378	2209	4694	3161	1998	1396	2066	1391	1337	934	1796	1175
K	3933	704	6094	1395	7095	1085	4972	1533	4951	628	5780	849	739	dl
K(L)	4220	972	4620	596	5552	418	5407	712	5258	366	6494	472	6420	555
K (ave)	4076.5		5357		6323.5		5189.5		5104.5		6137		3579.5	
Mn	1.7	0.23	2.6	0.29	2.1	0.22	2.7	0.35	1.7	0.12	1.7	0.11	1.4	0.12
Sb(L1)	0.034	dl	0.028	dl	0.015	0.013	0.062	0.023	0.009	0.005	0.012	0.0048	0.007	0.002
Sb(L2)														
Br	2.3	0.63	1.8	0.51	23	6.2	6.1	1.6	4.8	1.3	1.4	0.39	1.7	0.46
Cd	0.19	0.095	0.081	dl	0.087	dl	0.098	dl	0.014	0.011	0.011	0.0084	0.0037	0.0063
Na(L)	7500	484	9230	596	9633	622	7257	469	5296	342	5624	363	6021	389
Na(SH)	7100	480	9600	650	9600	640	7200	480	5600	380	5400	360	5200	350
Na(ave)	7300		9415		9616.5		7228.5		5448		5512		5610.5	
Cs	0.091	0.075	0.11	0.15	0.27	0.13	0.24	dl	0.14	0.056	0.038	0.026	0.098	0.014
Cr	2	0.53	15	0.46	4	0.38	19	0.45	2.1	0.16	5	0.15	0.93	0.054
Eu	0.063	0.026	0.085	dl	0.074	0.055	0.033	0.033	0.039	dl	0.028	dl	0.0017	0.0025
Fe	114	dl	206	69	232	63	133	44	80	29	99	21	119	6.7
Rb	2.4	2.2	5.3	dl	8.4	dl	11	3	12	1.8	2	dl	2.8	0.42
Se	0.541	0.211	1	dl	0.4	0.35	0.66	0.35	0.53	0.25	0.42	0.18	0.43	0.1
Zn	32	5.2	24	5.1	28	5.7	29	4.5	20	2.4	23	2.5	18	1.9

	J20	+/-	J21	+/-	J22	+/-	J4	+/-	J8	+/-	J12	+/-	J16	+/-
	ASC8		ASC9		ASC10		CSC1		CSC2		CSC3		CSC4	
Mg	525	101	188	102	455	102	492	87	1649	321	488	88	715	132
Al	0.73	2.3	14 dl		8 dl		15	4.6	30 dl		1.9	2	8.5 dl	
Ti	9.2	9.3	15	18	10	6.3	15	9.4	119 dl		7.3	7.5	14	12
V	0.043	0.05	0.4 dl		0.27 dl		0.12	0.078	0.071	0.25	0.12	0.072	0.23 dl	
Cl	1857	1214	3319	2171	2581	1688	1785	991	14660	9880	2130	1127	3544	2390
K	6947	1021	8074	1358	5967	1114	5421	615	41210	4620	4200	553	4785	595
K(L)	9232	1201	3983	373	4599	335	6314	454	41930	4360	4425	397	5838	404
K (ave)	8089.5		6028.5		5283		5867.5		41570		4312.5		5311.5	
Min	2.1	0.14	3	0.3	1.4	0.23	1.5	0.099	6.2	0.39	1.4	0.12	1.4	0.13
Sb(L1)	0.012	0.003	0.017 dl		0.019	0.009	0.013 dl		0.013	0.0053	0.019 dl		0.012 dl	
Sb(L2)														
Br	3.2	0.85	3	0.81	5.3	1.4	2.3	0.6	21	5.6	1.5	0.41	2	0.52
Cd	0.025	0.013	0.043	0.021	0.011	0.021	0.035 dl		0.039	0.025	0.026	0.018	0.035 dl	
Na(L)	6303	407	8660	559	7225	466	7129	460	29030	1870	6665	430	7901	510
Na(SH)	5400	370	15000	1040	7400	500	6300	420	45000	3000	5800	380	7800	520
Na(ave)	5851.5		11830		7312.5		6714.5		37015		6232.5		7850.5	
Cs	0.057	0.011	0.11	0.058	0.17	0.096	0.044	0.092	0.051	0.021	0.087	0.045	0.12 dl	
Cr	1.2	0.053	5.1	0.21	1.4	0.37	2.2	0.19	4.3	0.15	5.3	0.19	2.9	0.13
Eu	0.0037	0.0018	0.029	0.016	0.095	0.032	0.043 dl		0.016 dl		0.057 dl		0.046 dl	
Fe	80	4.8	132	25	56	42	70	26	558	26	95	33	83	24
Rb	6.9	0.61	4.4	1.8	4.4	2.5	6.7	1.8	14	1.4	11	2.9	9.5	1.8
Se	0.52	0.11	1.1	0.38	0.89 dl		1.1	0.37	4.7	0.71	0.67	0.22	0.86	0.25
Zn	22	2.3	26	3.2	20	4.4	25	3.7	132	15	28	3.6	31	3.7

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