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# Preparation, Analysis and Certification of PEPPERBUSH Standard Reference Material

Edited by Kensaku OKAMOTO

THE NATIONAL INSTITUTE FOR ENVIRONMENTAL STUDIES
環境庁　国立公害研究所

# Preparation, Analysis and Certification
# of PEPPERBUSH
# Standard Reference Material

Edited by
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Division of Chemistry and Physics
The National Institute for Environmental Studies

PEPPERBUSH PLANT
*(Clethra barbinervis* Sieb. *et* Zucc.*)*

# *Preface*

Measurement of the physical and chemical properties of substances is fundamental to the field of Material science. In order to understand the chemical properties and behaviour of material, knowledge of elemental composition in terms of accuracy and precision is required. With the latter objective in mind, standard reference materials of diverse categories have been prepared and issued by institutions in a number of countries for many years.

When our institute was inaugurated in 1974, the preparation of environmental standard reference materials was chosen as one of the long-term research projects for the Division of Chemistry and Physics. It is my great pleasure, therefore, to write this introductory note on "Pepperbush", the first standard reference material, to be issued by the institute.

Dr. K. Okamoto, of the Biochemical Analysis section, was chief investigator of the project together with Dr. Y. Ambe, head of the Air Quality and Biochemical Analysis sections. Dr. C. McLeod, visiting researcher from the United Kindgom, undertook ICP analysis of Pepperbush while Dr. T. Takamatsu, a member of the Division of Water and Soil Environment, provided results by X-ray fluorescence spectrometry. Dr. Murozumi, Professor of Muroran Institute of Technology, gave a special contribution in providing results by isotope dilution mass spectrometry. The isotope dilution technique is indispensable for this kind of work and we are most grateful for the efforts of Dr. Murozumi. A special thanks is also due to Professor T. Kiba and his research group for including Pepperbush as one of their sample materials within the special environmental research programme which was supported by the Ministry of Education.

September, 1980.

Keiichiro Fuwa
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# CHAPTER I

# STANDARD REFERENCE MATERIALS IN ENVIRONMENTAL ANALYSIS

Y. Ambe

## *Introduction*

In recent years, an increasing number of chemical analyses of various kinds of environmental samples, such as the atmosphere, water, soil and biological materials, have been carried out in laboratories of universities, national and local governmental organizations, and independent institutions. The main purpose of such analyses are to indicate the actual state of environmental pollution, detect the onset of adverse changes in the environment and to understand the origin and mechanisms of the phenomena. The pollutants taken as objects for environmental monitoring include not only those controlled legally as toxic substances but also such chemicals that are potential hazards. In order to characterize the distribution of pollutants in the environment, and/or to detect abnormal values in a time series over a long period, a large number and a wide variety of environmental samples are collected and analyzed. Unfortunately the reliability of data provided by laboratories engaged in such projects is open to question since there is a lack not only of appropriate standard reference materials but also reference methods of analysis. In many instances, therefore, it is not possible to properly assess the analytical data and to decide whether the data constitutes an environmental problem. It is mainly for this reason that the National Institute for Environmental Studies (NIES) has recently instigated a standard reference material's (SRM) programme. This introductory chapter describes the chemical characteristics of environmental samples and discusses the concept of the SRM within the framework of environmental analysis. The status of the SRM programme at NIES is also summarized.

## *Characteristics of Environmental Samples*

First, we should consider the characteristic properties of environmental samples from the view point of chemical analysis. Here, environmental samples include air, water, soil, plant and animal tissue, containing or not containing pollutants directly hazardous to our health, and include those thought of as indicators for monitoring environmental pollution.

### 1) Multicomponent systems

Environmental samples are composed of many elements and compounds, and sometimes different phases co-exist. The complex composition of the materials may inhibit accurate analysis of the objective components. For example, the co-existence of major elements may sometimes cause matrix interference, increasing or decreasing the value under measurement. Also the chemical composition of environmental samples differ greatly so that the appropriate analytical method should be selected considering the matrix of the analyte. Very often the analytical procedure requires a chemical separation of the component of interest from the original material.

### 2) Heterogeneous composition and distribution of pollutants

Biological and geological samples, characteristically, are heterogeneous in composition and pollutants may be distributed heterogeneously according to their physico-chemical properties. In animal body, halogenated pesticides like BHC have a tendency to concentrate in the fatty part of the body, and, therefore, concentrations differ according to the sites analysed. It is also important to specify whether results are expressed on a "total" or "fat" basis. In this case, the units to express the concentrations should be carefully selected to provide the most meaningful results.

When we intend to express concentrations on a total basis, complete homogenation of the sample is necessary prior to analysis. It is rather difficult, however, to prepare homogenous samples for some types of material such as human hair or fish.

### 3) Wide range of concentrations

Concentrations of pollutants may vary from ppt levels to several hundreds of ppm so that the selection of an appropriate analytical method for the concentration level expected is an essential point.

For the determination of low level concentrations, the handling and pretreatment of sample must be carefully performed taking care to prevent contamination or loss. Special facilities such as a clean laboratory are of value to minimize contamination from air particulates. Reference methods of analysis for determinations at the ppb or ppt level are required and, in spite of many efforts, few such methods have been established.

### 4) Complex chemical forms of components

It is well known that the essentiality and toxicity of a element are closely related to not only the total amount of a metal but also the chemical forms of the element. An important requirement, therefore, is that analysis involving chemical speciation should be performed to evaluate the quality of our environment correctly.

The chemical forms of an element may be exceedingly complex, and the existence of various oxidation states, and chemical binding to low molecular weight compounds such as a metallo-organic substance and/or high molecular weight compounds such as a metalloprotein should be considered. The identification and determination of each chemical species invariably requires a preliminary separation of the component of interest prior to determination.

The determination of total amounts of elements has been extensively carried out for environmental studies, but it is much more difficult to determine the chemical forms of elements. The development and improvement of separation techniques which cause little denaturation of the *in vivo* components are required.

This situation is also true of organic compounds which have many derivatives.

### 5) Stability of samples

During transportation of the sample from the collection point to the laboratory substantial changes in the amounts and chemical forms of pollutants and sample media may occur. Care must be taken to select the appropriate sampler, container and preservation procedure. Generally environmental samples can readily undergo physiological and chemical changes due to microbial activity. Therefore, the most suitable preservation methods such as acidification, freezing or sterilization should be applied to the sample.

As mentioned above, many features of environmental samples make accurate analysis difficult and the proper selection of the analytical methods and techniques is required. Analytical methods should be selected considering not only the nature of the sample but also sensitivity and inherent accuracy of the analytical technique. The facility and instruments of laboratory and the experience of a scientist are also factors which determine the analytical methods and techniques to be used.

### *Use of Standard Reference Materials*

The various application areas of SRMs in measurement systems have been discussed extensively in a NBS booklet1) and are briefly summarized below:

(1) The SRM can be used as a standard to calibrate a measurement system. Namely, by analysing a SRM using a specified method or technique, the accuracy of the analytical method can be checked.

(2) In developing a new analytical method the accuracy and precision of the method have to be judged. SRMs can, therefore, serve to establish the reliability of the new method.

(3) The SRM can be used to assure long-term quality control particularly for routine and field applications by analysing the SRM at appropriate and regular time intervals. In this case, the preservation of the SRM should be guaranteed. In the field of environmental science, quality control for governmental and local legislation is of vital importance. As mentioned earlier a large number of data on a wide variety of environmental samples have been reported from many laboratories, but these data sometimes lack an assurance of accuracy since data have been obtained by different analysts of diverse experience and by different analytical methods. Thus, the value of the SRM in this area is clearly recognized.

### *Requisites for Standard Reference Materials*

The following items are considered to be requisites for the SRM.

**(1) Certified material**

It is a fundamental point that the analytical values of the components are well-characterized and certified. Such numerical values are termed "certified values" and are considered to be the most accurate within the limitations imposed by equipment and methodology. Adequate information on the certified values, the uncertainties of the values and instructions on sample preparation should be specified. Details of the certification process for Pepperbush are given in Chapter VIII.

**(2) Demand for material**

SRMs were orignially made in fields of industry and commerce, for example, to assist in measurement and exchange of goods1). In recent years several categories of environmental SRMs have been prepared in order to improve the quality of environmental analysis. It is not unreasonable, therefore, that biological and environmental SRMs have been issued by the National Bureau of Standards, U.S.A. (NBS) and other governmental organizations since the 1970's to meet the growing demands of environmental scientists.

**(3) Homogeneous and stable material**

The homogeneity of composition and stability of each aliquot or bottle of sample must be guaranteed. By definition the SRM is a homogeneous and stable material. Liquid or powdered samples are favorable for the preparation of a homogeneous material. In preparing a SRM, precipitates or large particles which cause inhomogeneity of the material should be removed by filtration or sieving. It is also essential that the structure, composition and properties of the SRM will not change over a long period so that the certified values, once determined, will be valid for more than ten years. Various treatments or storage methods such as sterilization by -ray radiation, germicide addition, or cold storage, etc. should be utilized for long-term storage of SRMs of low preservability. Containers for SRMs also should be carefully chosen so that changes of content by contamination or adsorption loss do not occur.

**(4) SRM composition**

It is preferable that the composition of the SRM should be close to that of the sample being analysed, otherwise it may not be possible to make judgements on the reliability of analysis. The potential errors on trace element determinations caused by major matrix components is one area which should be considered when assessing data.

**(5) Long-term supply**

A sufficient quantity of the SRM should be prepared to supply users' needs for at least 5 - 10 years. Speedy distribution through an appropriate organization is also required for end-users to receive SRMs as soon as possible.

**(6) Availability at low cost and safety for transportation**

### *Historical Background and Present Status of Environmental SRM Programme at NIES*

SRMs were first prepared in 1904 by NBS; the metal samples being issued in chip form. Much later, in 1955, the U.S. Geological Survey (USGS) prepared standard rock samples, G-1 and W-1, which were distributed to analytical laboratories throughout the world. In the 1960's some studies concerned with "soft" standards were performed, in addition to continued issue of "hard" SRMs such as steels, glass, and cement, etc. Bowen's study on Kale powder began in 1960 and in this investigation, 42 elements were determined at many laboratories using 11 different analytical methods2). The metal content of a bone-ash standard was studied by Vallee3). Since 1970 a series of biological and environmental SRMs have been issued by NBS. The National Bureau of Standards SRM categories include "Orchard Leaves", "Bovine Liver", "Tomato Leaves", "Spinach", "Pine Needles", "Oyster Tissue", "River Sediment", "Air Particulate Matters", "Trace Elements in Water" and so forth. The International Atomic Energy Agency has also distributed some certified reference materials or reference materials such as "Soil", "Lake Sediment", "Dried Copepoda", "Homogenized Fish Flesh" and etc.

In Japan, only two kinds of standard sample have been utilized in the environmental science field, one is a sea water sample for salinity measurement and the standard is calibrated versus the International Standard Sea Water prepared by the Institute of Oceanographic Sciences at Wormley, England, while the other is standard rock samples, JB-1, JG-1 prepared by the Geological Survey of Japan. Research materials such as tea leaves, shark meat, bamboo, and fungal mycelia, have recently been studied as candidates for SRMs4,5).

NIES has recently instigated a SRM programme, the objective being the preparation and certification of environmental standards, to serve the needs of environmental scientists and laboratories. Various categories of SRMs can be considered, for example, elements, gases, organic compounds. At present, our SRM programme is restricted to the preparation of environmental samples and certification of elemental composition.

**Table I**

NIES SRMs

| | | |
|---|---|---|
| Pepperbush | (Botanical) | Certified |
| Pond Sediment | (Geological) | Analysis in progress |
| Chlorella | (Biological) | Analysis in progress |
| Human Serum | (Clinical) | Analysis in progress |
| Hair | (Clinical) | Analysis in progress |

Table I shows the present status of the SRM programme at NIES. The first SRM to be completed is a botanical sample, Pepperbush, the preparation, analysis and certification of which are described in this book. Reports on the remaining SRMs will also be issued in due course. The most typical characteristics of Pepperbush relative to the botanical SRMs issued by NBS are the high concentrations of manganese, zinc, cobalt, nickel and cadmium.

The second issue is a geological sample, Pond Sediment, the certification of which is presently being performed. Sanshiro pond within the grounds of the University of Tokyo, was selected as the site for collecting the sediment. In May 1977, when the pond was being dredged during a cleaning operation, large amounts of salvaged sediment (about 500 kg, wet wt.) were taken and transported to the institute. After removal of coarse material larger than 2 mm, the sediment sample was collected on filter paper and was air-dried for about two weeks. The procedure after this step was almost the same as that for Pepperbush SRM, except for the mesh sizes used in sieving. The sediment samples having been passed through a 200-mesh (71 $\mu$m) nylon sieve were homogenized in a riffle sampler and then packaged in 2000 glass bottles (20 g each). The samples in packaged form were sterilized by $^{60}$Co radiation at 2 Mega-Rad at the Japan Atomic Energy Research Institute (Takasaki). The variation of metal content among bottles (estimated by AAS and XRF determinations) was negligibly small, indicating that the prepared pond sediment samples are homogeneous and practically useful as a SRM. Also, the comparison of metal contents between Pond Sediment and Palace Moat Sediment (Tokyo)[6] shows that the compositions are similar and, therefore, Pond Sediment SRM may be considered as a typical sediment sample.

The third SRM is a biological sample, Chlorella, the preparation of which has just been completed. Chlorella is a typical green algae which is widely distributed in lakes, rivers and ponds. In recent years, environmental pollution of the aqua system by heavy metals and organic compounds is having adverse effects on animals, plants, fish, and also on human health. An urgent requirement, therefore, is to identify and monitor such substances in the hydrosphere. Besides its value for monitoring water pollution, Chlorella SRM will also be of use in basic studies since chlorella has already been used extensively as a research material.

It is well known that certain inorganic elements are required for normal growth and development of the human body and, at present, about 26 elements are considered to be essential or beneficial for life. Diseases have been related to abnormal concentrations (deficiencies and excesses) of major, minor and trace elements in fluid or tissue. It is clear that there is a great need for human fluid and tissue SRMs and, therefore, a human blood serum SRM has been prepared and analysis is in progress. Human hair is also an important sample for monitoring metal accumulation in the human body and such a standard is presently being prepared. It should be realized that concentrations levels for many essential/non-essential elements in clinical samples are extremely low and reliable methods of analysis are not as yet established, and thus the certification of clinical SRMs is by no means an easy task.

As mentioned earlier, the SRM programme at NIES has been oriented to the preparation of diverse environmental samples and certification of elemental composition. Clearly there is a parallel need for SRMs where the contents of various organic compounds have been certified. Although there exist many difficulties with respect to the preparation, homogeneity, preservation, and analysis of the "organic" SRM, research for making such SRMs is to be carried out.

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# CHAPTER II

# NATURE OF PEPPERBUSH PLANT

K. Okamoto

## *Introduction*

Pepperbush (*Clethra barbinervis* Sieb. *et* Zucc), a deciduous tree of 3-7 m in height, is widely distributed in forested regions of Japan. From the biogeochemical and biochemical points of view, Pepperbush has two anomalous properties which characterize this tree from other normal plant species. First, the ability of the plant to accumulate certain heavy metals in the leaves has been established[1-3]. Analytical data on the metal contents of the plant and other species are presented in section 1. The chemical forms of the metals in pepperbush leaves and the molecular weights of the species have also been investigated and are discussed in section 2. In particular, the chemical form of copper, and the purification and properties of a copper protein isolated from pepperbush leaves have been studied and the results are described in section 3. The unusual ability of the plant to accumulate certain heavy metals also poses the question of whether high levels of certain metals are required for normal growth of the plant. The essentiality and toxicity of cobalt for pepperbush and the intercellular metal interactions between cobalt and other elements have been examined and part of the results are presented briefly in section 4.

The accumulation of heavy metals in pepperbush has nutritional significance since fresh leaves are edible[2, 4]. In olden times, young leaves were stockpiled by order of the law as a precaution against famine. The plant also provides a source of food for wild animals. These facts indicate that pepperbush leaves can supply cobalt and other essential trace metals which are required for normal growth and development.

Further, it is known that pepperbush plant grows well in areas of volcanic activity and also near ore refineries, suggesting that pepperbush plant is tolerant to exposure to sulfur compounds. Biochemical research to clarify the mechanism of adaptation has been performed in the Environmental Biology Division of the institute[5]. The results indicate that pepperbush plant occupies a unique position in the plant kingdom with respect to environmental pollution by sulphur compounds.

So far the botanical SRM s which have been issued are characterized only in terms for elemental composition. There is a need, however, for knowing not only the total amount of a particular element but also the chemical form. It is anticipated that there will be a demand in the near future for ranges of SRM s which provide information on the chemical form of elements.

### *1. Pepperbush, a Metal-accumulator Plant.*

Some plants which selectively accumulate elements such as copper, zinc and selenium have been extensively studied$^{6)}$, but little is known about the accumulation of cobalt by higher plants. *Clethra barbinervis* (pepperbush) was found by Yamagata and Murakami$^{1)}$ to accumulate cobalt in investigations on the variation in the heavy metal content of vegetation. More than one hundred times as much cobalt and several times as much nickel were detected in the leaves of *C. babinervis* as in other plant species$^{1, 2)}$.

Table I shows the analytical data on the heavy metal content of five native plant species growing at the same site$^{3)}$. The manganese content of the leaves of *C. barbinervis* ranged from 1190 to 2600 ppm ($\mu$g/g dry weight), being markedly higher than those of other plants. The zinc content of *C. barbinervis* ranged from 360 to 440 ppm, whereas those of other plants were between 28 and 72 ppm. The zinc content of land plants grown on normal soil is seldom in excess of 100 ppm, so that the value for *C. barbinervis* was exceptionally high. *C. barbinervis* had an extremely high cobalt content, ranging from 8 to 32 ppm. In contrast, those of the other four plants were less than 0.5 ppm. From three to seven times as much nickel were also found in *C. barbinervis* compared to other species. It is noteworthy that *C. barbinervis* contained extremely high levels of cadmium, ranging from 6.6 to 8.0 ppm. In the samples collected from Ginzandaira, *C. barbinervis* contained between about thirty and eighty times as much cadmium as *Q. serrata* and *Q. mongolica* var. *grosseserrata*, respectively.

On the other hand, the amounts of iron, copper and lead in *C. barbinervis* were not significantly different from those of the other four species, as shown in Table I. The data on the heavy metal content in various plant species shows that *C. barbinervis* accumulates manganese, zinc, cobalt, nickel and cadmium in the leaves, whereas there is no particular affinity for iron, copper and lead.

### *2. Chemical Forms of Some Metals in Pepperbush Plant*

It is of interest to study the chemical forms of the metals accumulated by pepperbush plant but as yet little is known about the subject. So far the presence of a vitamin $B_{12}$-like cobalt-porphyrin complex has been indicated by bioassay using microbes$^{2)}$. The purification of various metal-bind compounds from pepperbush leaves, therefore, has been performed in our laboratory over the past few years.

In order to examine the chemcial forms of metals in pepperbush, the buffer extract from pepperbush leaves was applied to a gel chromatographic column in order to estimate approximate molecular weights of metal compounds. Fresh pepperbush leaves (250 g) were ground with 50 mM phosphate buffer (pH 6.8) in a mechanical mortar for 30 min. The slurry was centrifuged at 18,000 x *g* for 20 min and then the supernatant was lyophilized. The lyophilized powder was dissolved (100 mg/ml) in 10 mM $MgCl_2$ and then 1 ml of the sample solution was applied to a Bio-Gel P-4 column (1.6 x 40 cm) which was equilibrated with 50 mM phosphate buffer (pH 6.8) containing 10 mM $MgCl_2$.

The gel chromatographic elution pattern (fraction range: m.w. 800 - 4000) of the extract and the molecular weight standards using Bio-Gel P-4 are given in Fig. 1. The upper graph indicates the elution patterns of Blue Dextran (void volume, m.w. 2 x $10^6$) , vitamin $B_{12}$ (m.w. 1350), $Zn^{2+}$ and $Co^{2+}$ which were used as calibration standards. For the middle graph, the protein concentration was determined at 280 nm and also by the method of Lowry; the concentrations of Cu, Co and Cd for each fraction were determined by atomic absorption. The lower graph shows the elution pattern of Zn, Mn and Fe

**Table I.**

Heavy Metal Contents of the Leaves of Various Plants Collected from Three Sites3)

| Site | Species | Metal content (μg/g dry weight) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mn | Zn | Co | Ni | Cd | Fe | Cu | Pb |
| Ginzandaira* | *C. barbinervis* | 1350 | 360 | 8.0 | 6.4 | 8.0 | 120 | 12 | 1.2 |
| | *Q. serrata* | 320 | 30 | <0.5 | 2.2 | 0.27 | 61 | 12 | 0.57 |
| | *Q. mongolica* | 350 | 28 | <0.5 | 2.4 | 0.10 | 42 | 12 | 0.25 |
| | *P. cuspidatum* | 78 | 56 | <0.5 | 0.9 | 0.50 | 110 | 9.7 | 0.70 |
| | *A. yokoscense* | 86 | 44 | <0.5 | 1.4 | 2.0 | 170 | 14 | 2.0 |
| Mikouchi* | *C. barbinervis* | 2600 | 420 | 32 | 12 | 7.6 | 230 | 19 | 6.1 |
| | *Q. serrata* | 650 | 67 | <0.5 | 3.8 | 0.76 | 120 | 17 | 2.7 |
| | *Q. mongolica* | 520 | 66 | <0.5 | 3.3 | 0.27 | 120 | 13 | 3.3 |
| Nikko** | *C. barbinervis* | 1190 | 440 | 19 | 5.6 | 6.6 | 110 | 21 | 5.0 |
| | *Q. serrata* | 380 | 46 | <0.5 | 2.2 | 0.20 | 77 | 20 | 2.7 |
| | *Q. mongolica* | 240 | 40 | <0.5 | 2.2 | 0.14 | 120 | 25 | 2.7 |
| | *P. cuspidatum* | 170 | 72 | <0.5 | 1.0 | 1.4 | 150 | 30 | 6.5 |
| | *A. yokoscense* | 80 | 65 | <0.5 | 2.3 | 0.55 | 230 | 24 | 5.4 |

*C. barbinervis* : *Clethra barbinervis* Sieb. *et* Zucc.
*Q. serrata* : *Quercus serrata* Thunb.
*Q. mongolica* : *Quercus mongolica* Fisch. var. *grosseserrata* Rehd. *et* Wils.
*P. cuspidatum* : *Polygonum cuspidatum* Sieb. *et* Zucc.
*A. yokoscense* : *Athyrium yokoscense* Christ.

* Ashio district, Tochigi Prefecture.
** Nikko botanical garden, Tochigi Prefecture.

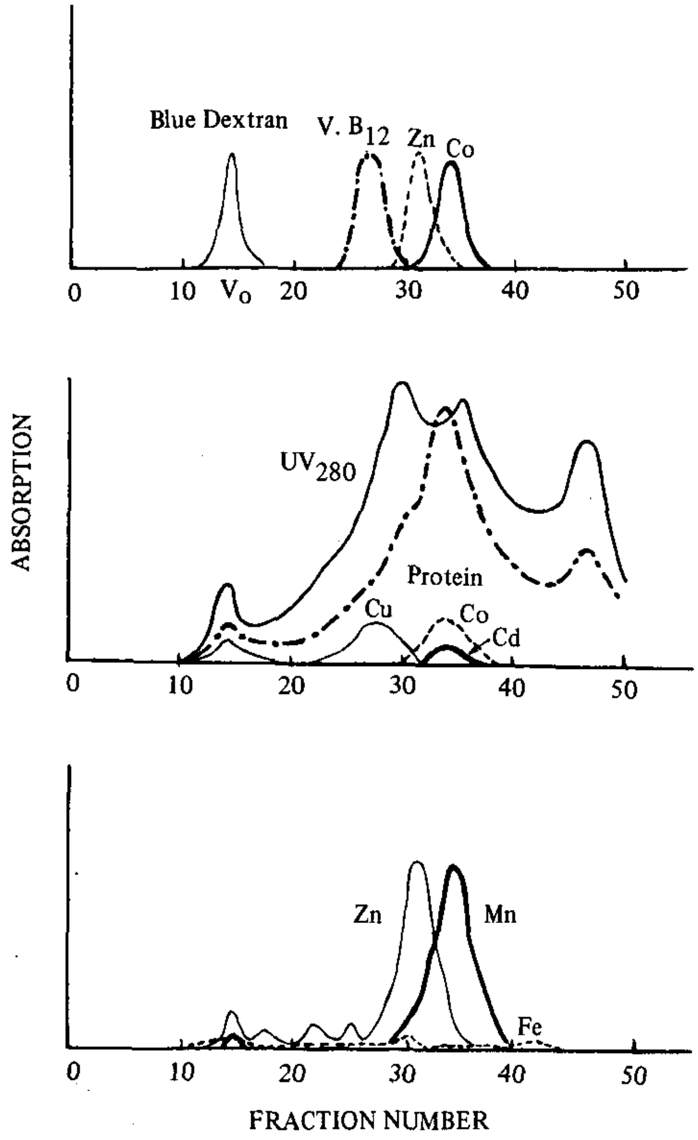

Fig. 1. Elution pattern of the buffer extract from pepperbush leaves on a Bio-Gel P-4 column (1.6 x 40 cm) equilibrated with 50 mM phosphate buffer (pH 6.8) containing 10 mM $MgCl_2$. High protein concentrations measured by Lowry's method (middle, fraction No. 30-50) are due to the reaction between Folin's reagent and the low molecular pigments. Blue Dextran,Vitamin $B_{12}$, solution absorption. Metals, atomic absorption. Solid line, 280 nm; Broken line, 650 nm.

extracted from pepperbush leaves on the column. Fig. 1 indicates that possible metals bound to high molecules (molecules which have molecular weight more than 4000 are eluted at void volume) include Cu and Zn, though most of the metals were detected at the elution volume of low molecular weight species. As for Co, Cd and Mn, the elution volume of the metals was almost the same as those of Co and Zn ions, suggesting that Co, Cd and Mn in pepperbush leaves are present as low molecular compounds or ions.

Further purification of cobalt-containing compound(s) was performed by ion exchange chromatography on a DEAE and a CM cellulose column but the presence of a single cobalt species has yet to be demonstrated. From the elution patterns of gel filtration and ion exchange chromatography, cobalt in pepperbush leaves appears to be present as a low molecular weight compound(s), possibly a cobalt-pigment complex, and/or as cobalt ions. Further studies are required to identify the chemical forms of cobalt in pepperbush leaves.

### *3. A Copper Protein (Plastocyanin) Isolated from Pepperbush Leaves*

As shown in Fig. 1, part of the copper in pepperbush leaves is bound to high molecular weight compounds. The purification of the copper protein was, therefore, performed and some properties of the isolated copper protein are described.

*Plant material.* Leaves of pepperbush were collected in August, 1977, at Mikouchi in the Ashio district, Tochigi Prefecture, where large amounts of the leaves are readily available$^{7)}$. The leaves were washed with deionized water and stored at -20 C until required.

*Purification.* Unless stated otherwise all steps were perfomed in the cold.

**Step 1.** *Extraction.* Frozen leaves of pepperbush (200 g) were cut into small pieces and ground in a mechanical mortar for 30 min with 50 g of polyvinylpyrrolidone (insoluble), 200 g of sand and 700 ml of 25 mM Tri-HCl buffer (pH 8.0) containing 1 mM dithiothreitol. The homogenate was centrifuged for 10 min at 3000 x *g* and the sediment was further ground for 30 min with 700 ml of the same buffer. After centrifugation of the homogenate, the supernatant solution was combined with that obtained by the first centrifugation. Fourteen additional batches of the leaves were similarly treated and the supernatant solutions were pooled (15 liters).

**Step 2.** *Ammonium sulfate fractionation.* The supernatant solution was brought to 30% saturation with solid ammonium sulfate. After standing for 1 hr, the precipitate was removed by centrifugation for 15 min at 10,000 x *g*. The supernatant solution was brought to 90% saturation by further addition of solid ammonium sulfate and left standing overnight. The precipitate was collected by centrifugation for 15 min at 10,000 x *g*, suspended in and dialyzed against 25 mM Tris-HCl buffer, pH 8.0. The dialyzate was clarified by centrifugation and brought again to 90% saturation with ammonium sulfate. The precipitate was collected by centrifugation, dissolved in and dialyzed against 25 mM Tris-HCl buffer, pH 8.0.

**Step 3.** *Stepwise DEAE-cellulose chromatography.* The dialyzed solution (370 ml) was applied to a column (2.6 x 35 cm) of DEAE-cellulose (Whatman DE-23), previously equilibrated with 25 mM Tris-HCl buffer, pH 8.0. The column was washed with the same buffer to give an eluate absorbance value of less than 0.1 absorbance units at 280 nm. Subsequently, the column was eluted with 400 ml of 25 mM Tris-HCl buffer, pH 8.0, containing 0.4 M NaCl. The protein fractions whose Cu concentrations were greater than 0.1 $\mu$g/ml were pooled and dialyzed against 25 mM Tris-HCl buffer, pH 8.0.

**Step 4.** *Linear gradient DEAE-cellulose chromatography* (Fig. 2). The dialyzed solution (180 ml) was charged to a column (1.6 x 30 cm) of DEAE-cellulose (Whatman DE-32), previously equilibrated with 25 mM Tris-HCl buffer, pH 8.0. Elution of the protein was

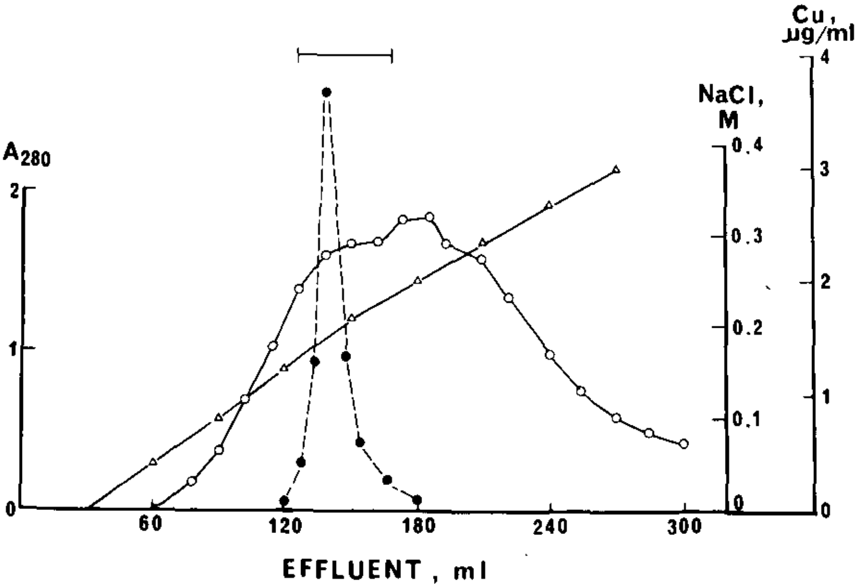

Fig. 2. Chromatography on DEAE-cellulose (DE-32). For details, see text.
○——○ , absorbance at 280 nm; ●- - - -● , copper (atomic absorption); △——△ , NaCl concentration.

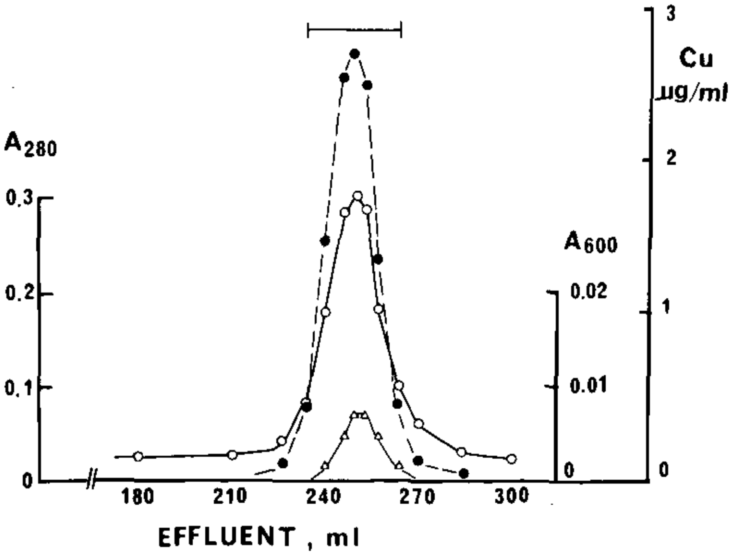

Fig. 3. Second Chromatography on Sephadex G-75. For details, see text.
○——○ , absorbance at 280 nm; △——△ , absorbance at 600 nm;
●- - - -● , copper (atomic absorption).

accomplished with a linear gradient prepared by mixing 100 ml of 25 mM Tris-HCl buffer (pH 8.0) containing 0.04 M NaCl with the same volume of 25 mM Tris-HCl buffer (pH 8.0) containing 0.4 M NaCl. As shown in Fig. 2, the copper protein was eluted at a concentration of about 0.2 M NaCl. The fractions indicated by the horizontal line (Cu concentrations greater than 0.1$\mu$g/ml) were pooled and dialyzed against distilled water.

**Step 5.** *Gel filtration on Sephadex G-75* (Fig. 3). The dialyzed solution was lyophilized and the resultant yellow-greenish powder was dissolved in 10 ml of 25 mM Tris-HCl buffer, pH 8.0. The solution was applied to a column (2.6 x 65 cm) of Sephadex G-75, previously equilibrated with the same buffer. During elution with the buffer, a faint blue band was observed under an orange-coloured band and this blue band corresponded to the peak of the copper protein. In this procedure the copper protein was not completely separated from the orange-coloured pigment. The fractions containing more than 0.1$\mu$g/ml of copper were, therefore, again applied to the column of Sephadex G-75 and eluted with the same buffer. As shown in Fig. 3, the absorption peaks, $A_{280}$ and $A_{600}$, and the copper peak were coincident. The fractions indicated by the horizontal line were pooled and used for analyses described below.

*Homogeneity.* Polyacrylamide disc gel electrophoresis gave a single protein band (Fig. 4).

*Molecular weight and subunit(s).*

1. The minimum molecular weight was calculated from the copper content. The protein contained 0.75% copper, which gave a minimum molecular weight of 8500.
2. The molecular weight was estimated by gel filtration on a Sephadex G-75 column (1.6 x 95 cm) equilibrated with 50 mM Tris-HCl buffer, pH 8.0. The standards used for calibration were cytochrome *c*, ribonulease, myoglobin, chymotrypsinogen and ovalbumin. The molecular weight of the copper protein was estimated to be approximately 17,000 (Fig. 5).
3. The molecular weight of the subunits of the protein was estimated by acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, with and without 2-mercaptoethanol (1% $wv^{-1}$). As shown in Fig. 6, the protein was not dissociated by sodium dodecyl sulfate unless mercaptoethanol was present. The protein, in contrast, was dissociated into two subunits of equal size in the presence of mercaptoethanol. Using cytochrome *c*, myoglobin, chymotrypsinogen and ovalbumin as the molecular weight standards, the molecular weights of the protein and the subunit were estimated to be about 20,000 and 10,000, respectively.

*Absorption spectrum.* The protein isolated by the procedure described above was faintly blue in color. The protein turned more intense blue by the addition of a few crystals of ferricyanide. The blue color was bleached completely by further addition of sodium ascorbate and a water-clear solution was obtained. As shown in Fig. 7, in the visible range, the protein in the oxidized form had a broad peak maximum at 600 nm. In the ultraviolet range it showed, besides the main absorption peak at 278 nm, the characteristic peaks at 259, 265 and 269 nm corresponding to the fine structure bands of phenylalanine, and a shoulder at 284 nm corresponding to that of tyrosine; the spectrum was very similar to those for spinach plastocyanin8) and tobacco plastocyanin9). The $A_{600}/A_{278}$ ratio of this copper protein was 0.25 and is much lower than those for spinach and tobacco plastocyanin ($A_{597}/A_{278}$ ratio of the purified plastocyanin are about 1.0).

*Metal contents.* The copper content of the purified protein was determined by non-flame atomic absorption spectrophotometry with a Perkin-Elmer 503 spectrometer fitted with a HGA 2000 atomizer. Similarly zinc, manganese and iron were determined by flame

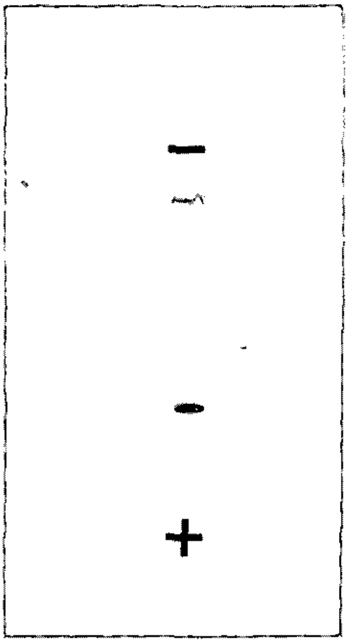

Fig. 4

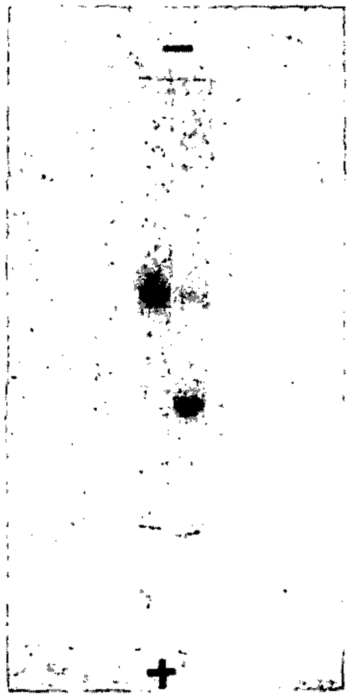

Fig. 6

Fig. 4. Polyacrylamide disc gel electrophoresis of the purified copper protein. The protein (50$\mu$g) was subjected to gel electrophoresis (gel 7.5%, pH 9.5) for 40 min at 4 mA per tube. The gel was stained with 1% amido black 10 B in 7% acetic acid and destained in 7% acetic acid.

Fig. 6. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the purified copper protein. The protein (10$\mu$g each) was subjected to electrophoresis on polyacrylamide gel (10%) in the presence of sodium dodecyl sulfate, with (right) and without (left) 1% 2-mercaptoethanol, as described by Weber and Osborn[11]. The gels were stained with 0.1% Coomassie brilliant blue (acetic acid, 1: methanol, 5: distilled water, 4) for 60 min and excess stain was removed using 7% acetic acid. It is apparent that dissociation into subunits was achieved only in the presence of 2-mercaptoethanol.

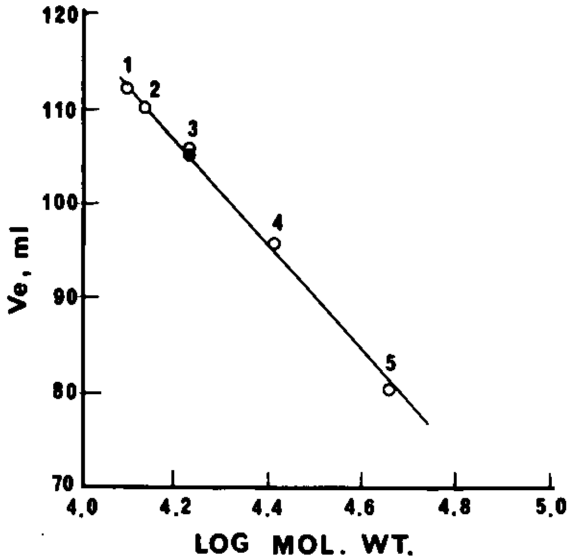

Fig. 5. Estimation of the molecular weight of the copper protein from pepperbush leaves by gel filtration on a Sephadex G-75 column (1.6 x 9.5 cm). The molecular weight standards used were: 1. cytochrome *c* (12,400), 2. ribonuclease (13,700), 3. myoglobin (17,000), 4. chymotripsinogen (25,800), 5. ovalbumin (43,000). The closed symbol (the elution volume of the copper protein) corresponds to a molecular weight of 17,000.

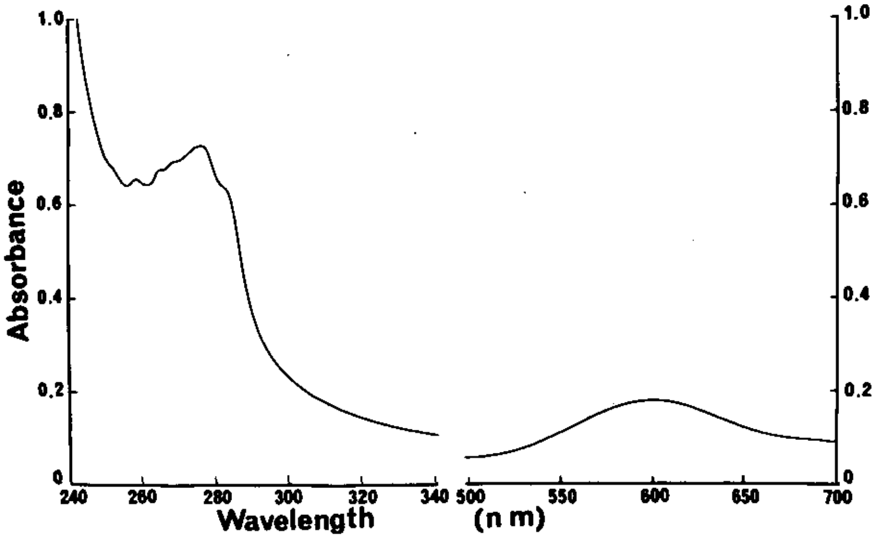

Fig. 7. Ultraviolet and visible absorption spectrum of the isolated and purified copper protein. The protein concentration was 0.69 mg/ml in 25 mM Tris-HCl buffer, pH 8.0. The absorption spectrum was recorded on a Hitachi 323 spectrophotometer.

atomic absorption spectrophotometry with a Hitachi 508A spectrometer. The results in Table II indicated that the protein contained two atoms of copper per molecule suggesting that one atom of copper was present per one subunit. No significant amount of zinc could be detected in the protein. The contents of iron and manganese were below the detection limits of the respective atomic absorption techniques.

*Enzymic activity.* Superoxide dismutase (EC1.15.1.1) activity was assayed according to the method of McCord and Fridovich[10)] but the protein did not exhibit enzymic activity.

*Amino acid analysis.* The copper protein was dialyzed against distilled water for a week. Triplicate samples of 0.7 mg of the protein were lyophilized in acid-washed tubes, dissolved in 1 ml of constant-boiling HCl and sealed under vacuum. Hydrolysis was accomplished at 110 ℃ for 24, 48 and 72 hr. Amino acid analysis was performed with a Hitachi 835 amino acid analyzer. The recoveries of serine and threonine were extrapolated to zero time. Valine and isoleucine were determined from the values at 72 hr of hydrolysis. Table III presents the result on amino acid analysis of the copper protein from pepperbush, and compares the result with those of spinach plastocyanin by Katoh *et al*[8)]. The amino acid composition showed considerable similarity to that of spinach plastocyanin, though the contents of aspartic acid, glutamic acid, glycine, methionine and lysine were lower.

**Table II.**

Metal Content of the Copper Protein Isolated from Pepperbush Leaves.

After the protein was dialyzed against distilled water for 2 days, the metal contents were determined by atomic absorption spectrometry and the protein concentration was determined by the method of Lowry *et al*[12)] using bovine serum albumin as the standard. The mole/mole ratio was obtained on the basis of a molecular weight of 17,000.

| | Metal content | |
|---|---|---|
| Metal | $\mu$g/g protein | g-atoms metal/mole protein |
| Cu | 7.5 | 2.0 |
| Zn | 0.25 | 0.06 |
| Fe | below 0.05 | below 0.01 |
| Mn | below 0.05 | below 0.01 |

**Table III.**

Amino Acid Composition

The amino acid composition of the copper protein and comparison to the data for spinach plastocyanin. All calculations were based on a molecular weight of 17,000 for the copper protein.

| *Amino acid* | Pepperbush copper protein | Spinach plastocyanin[8] |
|---|---|---|
| | Residues per molecule of protein | |
| Aspartic acid | 16.2 | 23.7 |
| Threonine | 6.9 | 10.6 |
| Serine | 15.0 | 15.8 |
| Glutamic acid | 12.6 | 23.4 |
| Proline | 9.6 | 9.2 |
| Glycine | 15.9 | 22.7 |
| Alanine | 9.3 | 13.0 |
| Valine | 15.6 | 15.2 |
| Methionine | 2.4 | 4.3 |
| Isoleucine | 3.9 | 3.5 |
| Leucine | 9.6 | 10.7 |
| Tyrosine | 3.9 | 4.2 |
| Phenylalanine | 9.5 | 9.7 |
| Lysine | 7.2 | 12.1 |
| Histidine | 3.2 | 4.1 |
| Arginine | 0 | 0 |
| Half-cystine | 2.1 | 2 |
| Tryptophan | - a) | 0 |

a) not determined.

### *4. Physiological Role of Cobalt for Pepperbush Plant.*

Another interesting physiological aspect revealed in Table I is the possible nutritional significance of the high cobalt content in pepperbush plant. A general requirement for cobalt by higher plants has not been established, except for nitrogen fixation by the symbiotic root-nodule bacteria[13, 14]. It is of interest, therefore, to study whether cobalt is essential for pepperbush plant or not. We tested for the essentiality, toxicity and uptake of cobalt by varying the amounts of cobalt added (none added to 10 ppm) to culture solutions. In addition, cobalt was added as either cobalt sulfate or vitamine $B_{12}$ to examine the effect of chemical forms of cobalt. Pepperbush plants grown from cuttings were cultivated in solution in a temperature-controlled greenhouse. Fig. 8 shows the photo of pepperbush plants after about 6 weeks' cultivation. It was found that the cobalt uptake by the plant remarkably depended on the chemical form of cobalt added, and that the contents of iron and manganese exhibited a marked dependence on the cobalt concentrations and the chemical forms of cobalt in the culture solutions. This work will be reported in detail elsewhere.

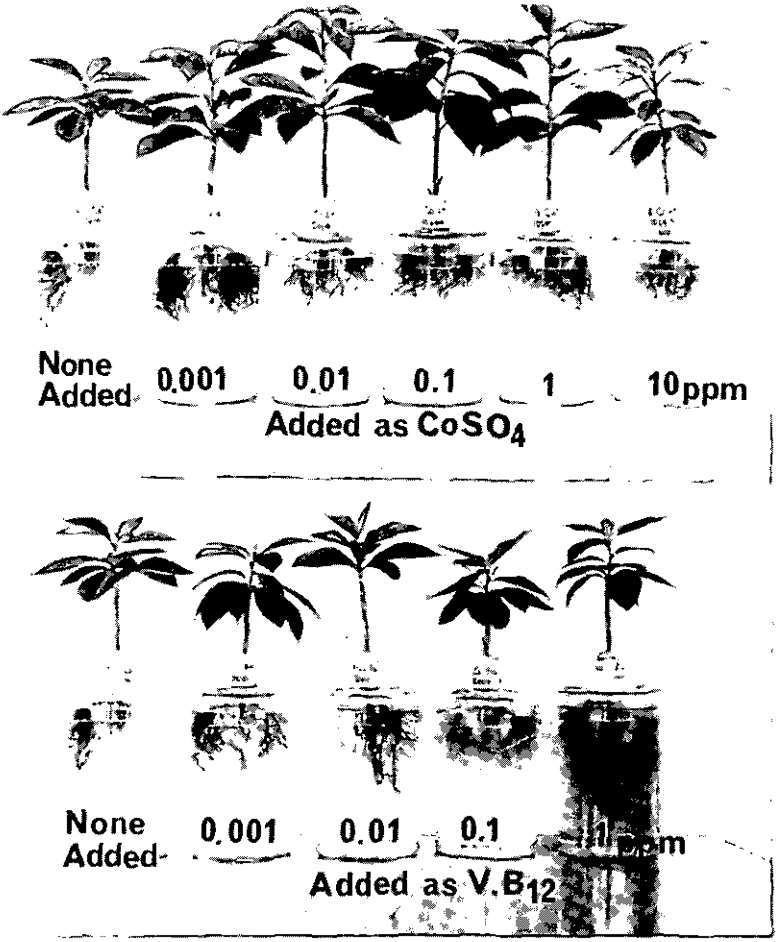

Fig. 8. Pepperbush plants in solution culture after about 6 weeks' cultivation. Pepperbush plants grown from cuttings were cultivated in the culture solutions[15] to which *cobalt was added as either cobalt sulfate* (upper series) or vitamin $B_{12}$ (lower series) with Co concentrations ranging from zero to 10 ppm.

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# CHAPTER III

# PREPARATION OF PEPPERBUSH

K. Okamoto

The preparation of Pepperbush consisted of the following steps.

*Sampling and Drying*

The leaves of pepperbush were collected in September, 1975, at Mikouchi in the Ashio district where large amounts of the plant are readily available. The leaves, free from stems, were washed with deionized water and dried in an air oven at 80 ℃ overnight. About 30 kg of the dry leaves were used in this work.

*Grinding and Sieving*

The dry leaves (about 700 g) were ground for about one hour in a ball-mill (95% $Al_2O_3$, 7 liters) which had been previously ground well with the leaves to minimize contamination by metal. The pulverized samples were placed on a set of sieves - a 50-mesh (297μm) nylon sieve (top), a 80-mesh (177μm) nylon sieve (middle) - and a reservoir made of vinylchloride (bottom), and then vibrated mechanically for 15 min.

*Distribution and Variation of Zn, Fe and Mn for Various Mesh Sizes*

To determine the distribution and variation of metal content for the samples divided into the three parts (above 50-mesh, from 50 to 80- mesh, below 80-mesh), five lots of the leaves (about 700 g each) were independently ground and passed through a set of the sieves. Three portions (1 g each) were taken from each of the three parts in five lots, dry-ashed and analyzed. Zn, Fe and Mn were determined by atomic absorption spectrometry using an air-acetylene flame.

**Table I.**
Distribution and Variation of Zn, Fe and Mn for Various Mesh Sizes (Lot No. 3)

| | Content [a], μg/g dry wt., | | | Rel.std.dev., % | | |
|---|---|---|---|---|---|---|
| | Zn | Fe | Mn | Zn | Fe | Mn |
| Above 50-mesh | 335 | 87 | 1950 | 2.6 | 3.2 | 1.2 |
| 50 to 80-mesh | 331 | 97 | 2030 | 1.5 | 2.3 | 0.3 |
| Below 80-mesh | 301 | 152 | 1950 | 0.5 | 0.5 | 0.5 |

*a* average value

Table I shows, for example, the analytical data on Lot No. 3 (the analytical data on the other four lots were almost the same). The finest part (below 80-mesh) contained less Zn and more Fe than the other two parts. Mn levels were almost the same for the three parts. These differences in the metal contents of the various fractions may be connected with the biochemical roles of the metals.

The relative standard deviations for the metal determinations are also given in Table I. The finest part showed the lowest values, indicating the best homogeneity, though the value of Mn is a little higher than that for the 50 to 80-mesh. The samples passed through a 80-mesh sieve were, therefore, selected for further processing.

### *Mixing*

The powder having passed through an 80-mesh sieve was divided into two parts with a riffle sampler (JIS No. 2) made of vinylchloride. The powder was piled up in two layers and again divided by passing through the riffle sampler. Pepperbush samples were homogenized by repeating this procedure ten times.

### *Effect of Mixing with a Riffle Sampler*

The homogeneity of the samples obtained by mixing with a riffle sampler was tested. Before mixing, three portions (1 g each) were taken from each of the five lots (320, 210, 420, 320 and 320 g), dry-ashed and analyzed. After the five lots were mixed by passing through the riffle sampler ten times, nine portions (1 g each) were taken from the mixed powder, dry-ashed and analyzed.

Table II shows the analytical data on the pepperbush samples before and after mixing. Before mixing, the relative standard deviations of Zn, Fe and Mn determinations in the five lots were 6.2, 12 and 13%, respectively. After mixing, those of Zn, Fe and Mn greatly decreased to 1.0, 3.1 and 0.7%, respectively. Good homogeneity was, therefore, achieved by mixing with the riffle sampler.

**Table II.**
Effect of Mixing with a Riffle Sampler

| | Before mixing | | | After mixing | | |
|---|---|---|---|---|---|---|
| | Zn | Fe<br>μg/g dry wt. | Mn | Zn | Fe<br>μg/g dry wt. | Mn |
| Maximum value | 340 | 152 | 2460 | 301 | 133 | 2070 |
| Minimum value | 290 | 110 | 1730 | 292 | 121 | 2040 |
| Average | 309 | 128 | 1990 | 296 | 127 | 2050 |
| Rel.std.dev., % | 6.2 | 12 | 13 | 1.0 | 3.1 | 0.7 |

### *Homogeneity of Pepperbush Samples on a Large Scale*

Seven lots (total 14.4 kg) of the pepperbush samples (below 80-mesh) were mixed in the riffle sampler (ten times) and the powder was randomly packaged in 1150 glass bottles (about 14 g each). To check the homogeneity of the pepperbush samples, 38 samples were taken from each of 30 bottles, dry-ashed and analyzed. Homogeneity was estimated from the relative standard deviations of Zn, Fe and Mn determinations of the pepperbush samples.

Table III shows that the relative standard deviations for Zn, Fe and Mn in the 38 samples were 1.5, 2.2 and 1.0%, respectively. When standard solutions of Zn, Fe and Mn at the same concentrations as the samples were measured ten times under similar conditions, the relative standard deviations were 1.4, 2.7 and 1.0%, respectively. The variation of the metal contents of the pepperbush samples were almost the same as the corresponding results for the standard solutions, indicating that the prepared pepperbush samples are homogeneous and practically useful as a standard reference material.

**Table III.**
Homogeneity of Pepperbush Samples

| | Zn | Fe<br>μg/g dry wt. | Mn |
|---|---|---|---|
| Maximum value | 345 | 172 | 2110 |
| Minimum value | 325 | 161 | 2020 |
| Average | 337 | 166 | 2090 |
| Rel.std.dev., % (38 samples) | 1.5 | 2.2 | 1.0 |
| Rel.std.dev., % *(std. solution 10 times)* | 1.4 | 2.7 | 1.0 |

## References

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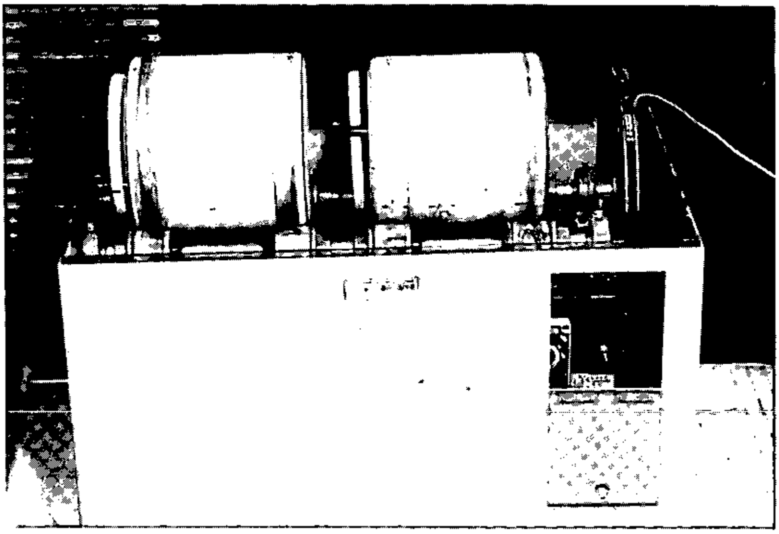
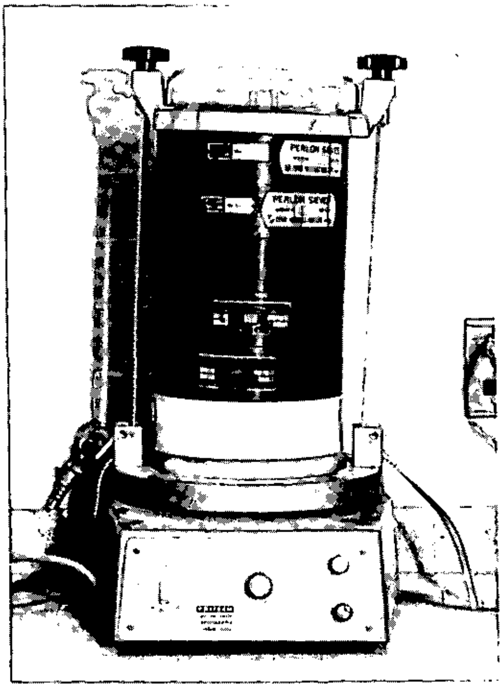
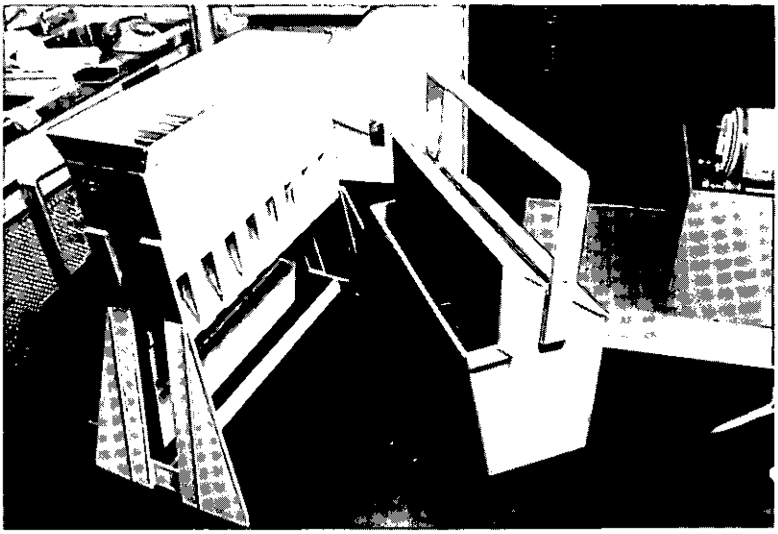
Okamoto, K. & Fuwa, K. (1979): Preparation of environmental standard reference materials. *Kankyo Sokutei Gijutsu* 6 (4): 1-6 (in Japanese).



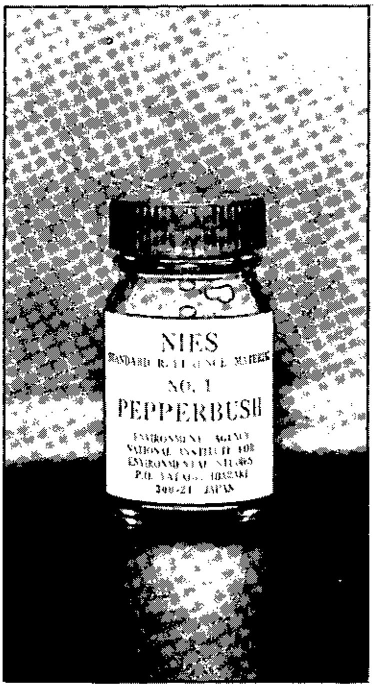
Alumina ball-mill



Sieving apparatus



Riffle sampler



Pepperbush Standard Reference Material

## CHAPTER IV

# ANALYSIS OF PEPPERBUSH BY ATOMIC ABSORPTION AND FLAME EMISSION SPECTROMETRY

K. Okamoto

### *Introduction*

Atomic absorption spectrometry (AAS) is a unique analytical technique for the determination of metallic elements and is used in many scientific fields. The acceptance of AAS is attributed mainly to the following factors: 1) high sensitivity for a large number of elements, 2) ability to control and overcome matrix interference, if present, 3) speed of analyses, rapid improvement in instrumentation, and so forth.

The atomic absorption technique can be grouped into categories: (a) flame AAS, (b) non-flame AAS, with respect to the mode of producing the atomic vapor. Historically, a flame has been the means of atomization for AAS and is still the basic source for the vast majority of AAS measurements, because of the advantages indicated above. However, disadvantages are that a relatively large quantity of sample is required and sensitivity is limited for many elements.

A relatively new technique for producing the atomic vapor is the non-flame devices. These devices can be grouped into categories such as (a) the cathodic sputtering technique (b) graphite furnace or carbon rod devices (c) tantalum ribbon and (d) the cold vapor method. These methods differ from flames in that the atomisation source is more conducive to the production of ground state atoms in contrast to the chemically reactive flame environment. Also, for the graphite furnace or carbon rod only a relatively small volume of the sample (1 to 50 $\mu$l) is necessary for analysis which gives these methods improved sensitivity over flame methods. However, the non-flame methods have the inherent disadvantage in that high precision is more difficult to attain.

The samples suitable for AAS analysis cover a wide range of materials and concentrations. The procedure for sample preparation depends on the type of samples, the elements to be measured and the precision and accuracy required, therefore, there is no general method for the preparation of all samples. Solution samples are nebulized directly to the flame but for solid samples a dissolution in hydrochloric, nitric or perchloric acids is first required. Proper pretreatment of samples is most important to perform AA analysis. The sample preparation for Pepperbush is, therefore, described here and also the analytical values for pepperbush are presented.

### *1. Sample Preparation for Determination of K, Ca, Mg, Mn, Zn, Fe, Na, Rb, Co, Cu, Ni and Cd.*

The following procedure* has been applied for dissolution of Pepperbush powder. A one

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* Adapted from T. Rains1).

or two gram sample, previously dried at 85 ℃ for 4 hrs in an oven, was transferred to a teflon beaker. Next, $HNO_3$ (20 ml, 1:1) was added and the system was left standing overnight. The beaker was heated on a hot plate (200 ℃) for about an hour, cooled and the sides of the beaker were rinsed with distilled water. After further addition of $HNO_3$ (10 ml) and HF (5 ml), the solution was evaporated to approximately 10 ml by heating. When black particles of unreacted carbon still remained, the addition of $HNO_3$ (10 ml) and heating were repeated. After the addition of $HClO_4$ (10 ml), the solution was heated to strong fumes of perchloric acid, cooled and the sides of the beaker were washed with distilled water. Heating was continued until the sample volume was reduced to approximately 2 ml. The beaker was left standing for about one hour after the addition of approximately 10 ml of distilled water. The digest was transferred to a 100 ml volumetric flask and diluted to volume with distilled water. The sample solution was stored in a 100 ml polyethylene bottle.

### 2. *Sample Preparation for the Determination of Pb.*

A closed digestion system illustrated in Fig. 1 was adopted for the determination of Pb. This system is preferred when dealing with volatile elements. One gram of Pepperbush powder (not dried in an oven) was placed in a flask (A) containing 5 ml of concentrated $HNO_3$ and left overnight. The flask was fitted with a condenser (B) and an acid-trap (C) to which 5 ml of 1 M $HNO_3$ was added. One ml of 30% $H_2O_2$ was added to the flask (A) each day for 4 consecutive days and gentle heating on a gas burner was continued until the digest became clear. The digest and the $HNO_3$ in the trap were quantitatively transferred to a 100 ml volumetric flask and the solution was made up to the mark with distilled water. The sample solution was subsequently filtered through a Millipore filter (25 mm, 0.45 $\mu$) which had been previously cleaned in 0.1 M $HNO_3$.

### 3. *Determination of K, Ca, Mg, Mn, Zn, Fe, Na, Rb, Co, Cu, Ni and Cd by Atomic Absorption Spectrometry Using Air-Acetylene Flame.*

#### Preparation of Standard Stock Solutions

Standard stock solutions (1000 ppm of each element) were prepared either by dissolving high purity metal powders in appropriate acids or by dissolving high purity metal-salts in distilled water, as shown in Table I.

#### Standard Working Solutions

A series of individual standard working solutions for Mn, Zn, Fe, Co, Cu, Ni and Cd were prepared by appropriate dilutions of the 1000 ppm stock solutions with 0.1 N $HClO_4$. The ranges of the standard working solution were chosen to encompass the concentrations in the Pepperbush solution. For Na and Rb determinations, K was added (final concentration of K was 150 ppm which corresponded to the K level in the sample solutions) to each of standard working solutions to compensate for ionization interference effects. For the determinations of K, Ca and Mg, the digests were further diluted fifty times with 0.1 M $HClO_4$. A series of mixed K, Ca and Mg solutions were used for standardisation.

**Table I.**

Standard Stock Solutions for Atomic Absorption Spectrometry*
1000 µg/ml (ppm) as the element in a final volume of 500 ml.

| Element | Procedure |
|---|---|
| K | Dissolve 0.9534 g KCl (99.999 %) in distilled water and dilute to volume. |
| Ca | Transfer 1.2487 g $CaCO_3$ (99.999 %) in 500 ml volumetric flask with 100 ml water, add 5 ml HCl, after $CO_2$ is released, dilute to volume. |
| Mg | Dissolve 500 mg Mg (99.9 %) in HCl and dilute to volume. |
| Mn | Dissolve 500 mg Mn (99.8 %) in $HNO_3$+HCl and dilute to volume. |
| Zn | Dissolve 500 mg Zn (99.99 %) in $HNO_3$ and dilute to volume. |
| Fe | Dissolve 500 mg Fe (99.98 %) in $HNO_3$+HCl and dilute to volume. |
| Na | Dissolve 1.271 g NaCl (99.999 %) in distilled water and dilute to volume. |
| Rb | Dissolve 0.7074 g RbCl (99.99 %) in distilled water and dilute to volume. |
| Co | Dissolve 500 mg Co (99.99 %) in $HNO_3$+HCl and dilute to volume. |
| Cu | Dissolve 500 mg Cu (99.99 %) in $HNO_3$ and dilute to volume. |
| Ni | Dissolve 500 mg Ni (99.99 %) in $HNO_3$ and dilute to volume. |
| Cd | Dissolve 500 mg Cd (99.99 %) in $HNO_3$ and dilute to volume. |

### Experimental Conditions

**Table II.**

Experimental Conditions for Determinations of K, Ca, Mg, Mn, Zn, Fe, Rb, Co, Cu, Ni and Cd in Pepperbush SRM by Atomic Absorption Spectrometry
Using Air-Acetylene Flame

| Element | Analytical line (nm) | Lamp Current (mA) | Slit Width (Å) | Flow Rate Air-Acetylene (l/min) | Burner Height (mm) | Background Correction |
|---|---|---|---|---|---|---|
| K | 769.9 | 7 | 3.8 | 10-2.5 | 4 | No |
| Ca | 422.7 | 8 | 3.8 | 10-2.5 | 10 | No |
| Mg | 285.2 | 5 | 3.8 | 10-2.4 | 5 | No |
| Mn | 279.5 | 5 | 1.9 | 10-2.6 | 4 | No |
| Zn | 213.8 | 6 | 3.8 | 10-2.4 | 4 | Yes |
| Fe | 238.3 | 9 | 1.9 | 10-2.5 | 4 | Yes |
| Na | 589.6 | 6 | 3.8 | 10-2.4 | 5 | No |
| Rb | 780.0 | 6 | 3.8 | 10-2.4 | 5 | No |
| Co | 240.7 | 9 | 1.9 | 10-2.5 | 6 | Yes |
| Cu | 324.8 | 7 | 3.8 | 10-2.3 | 4 | No |
| Ni | 232.0 | 9 | 1.9 | 10-2.3 | 5 | Yes |
| Cd | 228.8 | 5 | 3.8 | 10-2.3 | 3 | Yes |

Spectrometer : Shimadzu AA-640-12 atomic absorption and flame emission spectrophotometer.
Lamp : Hollow cathode lamp (Hamamatsu TV.)
Flame : Air-acetylene flame.

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* Adapted from J. Deans and T. Rains2).

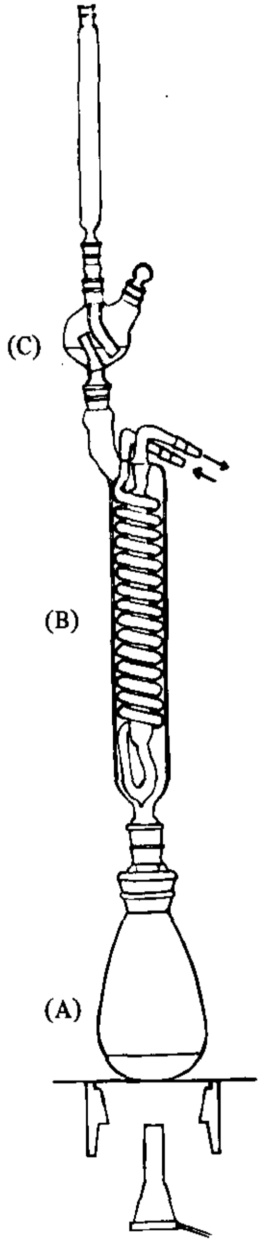

Fig. 1. Closed digestion system for the determination of lead and volatile elements.

### Results

One gram (triplicate, bottle No. 75) and two gram (triplicate, bottle No. 100) samples, dried at 85 ℃ for 4 hours in an oven, were digested with $HNO_3$, HF, and $HNO_3$ in teflon beakers as described in Chapter IV-1. Blank solutions (two) were prepared in the same manner. The digested and blank solutions were made up to 100 ml with distilled water.

**Table III.**

Analytical Results for Pepperbush by Atomic Absorption Spectrometry

| Element | Bottle No. 75 | | | Bottle No. 100 | | | Average±Std.Dev. |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 | |
| K* (%) | 1.52 | 1.52 | 1.51 | 1.54 | 1.53 | 1.55 | 1.53±0.01 |
| Ca* (%) | 1.31 | 1.30 | 1.30 | 1.31 | 1.32 | 1.33 | 1.31±0.01 |
| Mg* (μg/g) | 4130 | 4130 | 4180 | 4160 | 4170 | 4210 | 4160±30 |
| Mn* | 2110 | 2130 | 2080 | 2110 | 2080 | 2080 | 2100±20 |
| Zn | 351 | 341 | 344 | 342 | 331 | 333 | 340±7 |
| Fe | 201 | 212 | 201 | 215 | 204 | 198 | 205±7 |
| Na** | 99 | 101 | 102 | 99 | 101 | 102 | 101±1 |
| Rb | 75.5 | 76.0 | 79.4 | 73.1 | 71.7 | 74.5 | 75.0±2.6 |
| Co | 23.7 | 23.9 | 23.8 | 24.3 | 23.2 | 23.2 | 23.7±0.4 |
| Cu | 12.2 | 11.6 | 12.1 | 12.4 | 11.9 | 12.2 | 12.1±0.3 |
| Ni | 8.60 | 9.34 | 8.57 | 9.17 | 8.34 | 8.32 | 8.72±0.43 |
| Cd | 6.61 | 6.68 | 6.62 | 6.74 | 6.69 | 6.67 | 6.67±0.05 |

The values are based on the material dried at 85 ℃ for 4 hrs in an oven.

* 50 times diluted with 0.1 N $HClO_4$.

** After subtraction of the Na value for the blank solution.

## *4. Determination of Pb by Non-Flame Atomic Absorption Spectrometry*

### Sample Preparation and Standard Solutions

For the determination of Pb, one gram (triplicate each bottle, bottle Nos. 75 and 100) of Pepperbush was transferred to a flask and digested with $HNO_3$ and $H_2O_2$ in the system shown in Fig. 1. The digestion procedure has also been described in Chapter IV-2.

The standard stock solution (1000 ppm of Pb) was prepared by dissolving 0.7994 g of $Pb(NO_3)_2$ (99.9999 %) in a small volume of 0.1 M $HNO_3$ and diluting to 500 ml with 0.1 M $HNO_3$. The working solutions were prepared by appropriate dilution of the 1000 ppm stock solution with 0.1 M $HNO_3$.

### Experimental Conditions

Pb was determined by non-flame atomic absorption spectrometry with an IL-251 atomic absorption spectrophotometer equipped with an IL-555 atomizer. Background correction was performed using a deuterium hollow cathode lamp. Table IV shows the experimental conditions for Pb determination in Pepperbush.

**Table IV.**

Experimental Conditions for Determination of Pb

|  |  |
|---|---|
| Spectrometer | : IL-251 atomic absorption spectrometer equipped with IL-555 atomizer. |
| Lamp | : Pb hollow cathode lamp (Hamamatsu TV.), Deuterium hollow cathode lamp (Westinghouse) for background correction. |
| Lamp Current | : 5 mA for Pb H.C.L., 25 mA for deuterium lamp. |
| Analytical Line | : 283.3 nm. |
| Spectral Bandpath | : 1 nm. |
| Dry | : 75 ℃ for 15 sec., 100 ℃ for 15 sec. |
| Ash | : 400 ℃ for 20 sec., 550 ℃ for 20 sec. |
| Atomize | : 1900 ℃ for 10 sec. |

## Results

**Table V.**

Pb Content of Pepperbush.

The analytical values are expressed based on a dry weight at 85 ℃ for 4 hrs.

| Bottle No. | Sample No. | Pb content (μg/g) |
|---|---|---|
| 75 | 1 | 5.30 |
|  | 2 | 5.51 |
|  | 3 | 5.46 |
| 100 | 4 | 5.82 |
|  | 5 | 5.71 |
|  | 6 | 5.59 |
|  | Average±S.D. | 5.57±0.18 |

### 5. *Determination of K, Ca, Na, Rb, and Sr by Flame Emission Spectrometry*

**Sample Preparation and Standard Solutions**

The digestion of pepperbush samples was performed with $HNO_3$, HF and $HClO_4$ as described in Chapter IV-1. One gram samples were digested and diluted to 100 ml. For the determination of K and Ca, the solutions were diluted 50 times with 0.1 M $HClO_4$. To the standard working solutions of Na and Rb, K was added to give 150 ppm of K which corresponded to the K level in the sample solutions. Ca was added to each of the working solutions of Sr to provide the final concentration of 150 ppm Ca.

## Experimental Conditions

Table VI.

Experimental Conditions for the Determination of K, Ca, Na, Rb and Sr by Flame Emission Spectrometry

| Element | Analytical Line (nm) | Slit Width (Å) | Flow Rate Air-Acetylene (l/min) |
|---|---|---|---|
| K | 766.5 | 1.9 | 10 - 2.5 |
| Ca | 422.7 | 1.9 | 10 - 2.5 |
| Na | 589.0 | 1.9 | 10 - 2.3 |
| Rb | 780.0 | 1.9 | 10 - 2.3 |
| Sr | 460.7 | 1.9 | 10 - 2.4 |

Spectrometer : Shimadzu AA-640-12 atomic absorption and flame emission spectrophotometer.

Flame : Air-acetylene flame.

## Results

Table VII.

Analytical Results for Pepperbush by Flame Emission Spectrometry

|  | Bottle 75 |  |  | Bottle 100 |  |  | Average±S.D. |
|---|---|---|---|---|---|---|---|
| Element | 1 | 2 | 3 | 1 | 2 | 3 |  |
| K (%) | 1.54 | 1.53 | 1.54 | 1.54 | 1.53 | 1.52 | 1.53±0.01 |
| Ca (%) | 1.28 | 1.32 | 1.26 | 1.38 | 1.33 | 1.35 | 1.32±0.04 |
| Na ($\mu$g/g) | 106 | 110 | 107 | 102 | 106 | 105 | 106±3 |
| Rb | 76.2 | 71.8 | 75.1 | 71.1 | 76.6 | 77.4 | 74.7±2.6 |
| Sr | 28.1 | 32.0 | 29.0 | 29.0 | 29.5 | 32.0 | 30.1±1.6 |

The analytical values are expressed based on a dry weight at 85 ℃ for 4 hrs.

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1. Rains, T.C. (1977): Atomic absorption and flame emission spectrometry. A. Determination of beryllium, cadmium, copper, magnesium, manganese, mercury, nickel and zinc by atomic absorption, and of calcium, potassium, rubidium and sodium by flame emission spectrometry in biological and botanical materials. *NBS Special Publication* 492: 49-65.
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# CHAPTER V

# ELEMENTAL ANALYSIS OF PEPPERBUSH BY ICP EMISSION SPECTROMETRY *

C.W.M. McLeod, N. Furuta and M. Nishikawa

## *Introduction*

The standard reference material (SRM) certified for elemental composition is indispensable to analytical methods if accuracy and precision are to be achieved and maintained. To meet the ever-increasing demand from the many areas of science and industry, government-supported bodies in a number of countries are actively involved in the issuance of various categories of SRM's. In Japan, the National Institute for Environmental Studies (NIES) recently instigated an SRM programme to serve the needs of environmental scientists and the botanical reference material, Pepperbush, is the first standard to be issued. A report on the preparation of Pepperbush and comparison of elemental content with other botanical SRM's has been published1). Other categories of environmental SRM's already prepared include Pond Sediment and Chlorella and analysis is underway.

The certification process, in the first instance, requires analytical data for the elements to be obtained from independent and established analytical techniques. Inductively coupled plasma (ICP) emission spectrometry, a relatively new technique, has made a significant impact in the elemental analysis field2) and it is considered that it can make an important contribution to the SRM certification process. Noteworthy features of the ICP technique have been discussed in the literature2,3) and include the applicability to metals and non-metals, a high sensitivity for all elements with a single set of plasma operating conditions, a simultaneous multielement capability, a wide dynamic range and a relative freedom from interelement matrix interference. The primary objective in the certification process is the attainment of accuracy and from this point of view the relative absence of matrix interference is a particularly significant feature.

In this report, a description of the plasma emission technique and instrumentation at an elementary level is first presented and then consideration is given to the selection of plasma operating conditions for simultaneous multielement analysis (SMA). The various factors which may influence the accuracy and precision of ICP measurement are discussed. The analytical method is described in detail and the reliability of the results for Pepperbush is assessed by referring to the corresponding data for Orchard Leaves SRM (National Bureau of Standards, U.S.A).

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*Plasma Description and Instrumentation*

The inductively coupled argon plasma (ICP), a high temperature excitation source, is formed by the coupling of ionized argon with a powerful radio frequency (RF) field. When sample, usually in liquid form, is introduced to the plasma, as an aerosol mist via pneumatic nebulization, the aerosol particles undergo various processes such as desolvation, decomposition, atomization/excitation and ionization/excitation. The atomic and ionic emission characteristic of analyte elements is received by a spectrometer and the multielement signals are processed by a dedicated computer.

The experimental arrangement for plasma production consists, typically, of a 3-walled quartz tube coupled to a spray chamber and nebulizer. A copper induction coil, through which coolant water is passed, surrounds the top end of the quartz tube. A schematic diagram of a conventional system is given in Fig. 1. Two main argon gas flows, namely coolant (outermost~15 l/min) and nebulizer (innermost flow~1 l/min) serve to sustain the plasma (and prevent overheating of the torch) and to transport the sample aerosol to the plasma. Use of the auxiliary argon gas flow is optional. For initiation of the plasma, a tesla coil is used to ionize the argon gas stream and provide a "seed" of electrons which become thermally excited in the alternating RF field. A rapid rise in electron energy promotes further ionization of the argon and the collisional excitation processes that ensue develop and maintain the flame-like discharge. The sample aerosol gas stream penetrates the centre of the plasma and passes up through a narrow central axial channel. The high temperatures which the analyte experiences in the inert argon atmosphere and the relatively long analyte residence times render the ICP an exceptional excitation source for analytical emission spectrometry.

In this study, two independent ICP instruments are utilized, a conventional direct reader and a programmable monochromator system incorporating a silicon intensified target (SIT) detector. A brief description of equipment and operating conditions for both systems are given in Table I. The direct reader system is a commercially available instrument (Jarrell-Ash Atomcomp, Model 975) and has the capability of the simultaneous determination of 26 elements at sensitivity levels which are typically at the part per billion order. A schematic diagram of the main components of the instrument is given in Fig. 2. The polychromator, a 0.75 m Paschen-Runge unit, contains prealigned photomultiplier tube (PMT) detectors and respective exit slits situated along the focal curve at appropriate wavelength positions. The optical configuration is essentially fixed but extending element capability is possible by installing additional PMT's at the desired wavelength positions. The emission wavelengths for elements programmed into the system are given later. The instrumentation also features a single channel monochromator to provide for variable wavelength monitoring and wavelength scanning.

System operation, under computer control, is performed via the high-speed teletype device. Input commands are entered as a series of letters in order to perform standardisation and to implement the analysis sequence. The multielement emission signals after detection and processing are displayed on the teletype usually in units of concentration.

Novel research instrumentation incorporating a silicon intensified target (SIT) as the primary radiation detector has recently been developed[4]. The computer-controlled system employs the slew-scan technique whereby the monochromator is programmed to address, in sequence, only the desired emission wavelengths (wavelength regions of no interest are rapidly bypassed) to provide a rapid sequential multielement analysis capability. The experimental system is depicted in Fig. 3. Analysis procedures through appropriate software

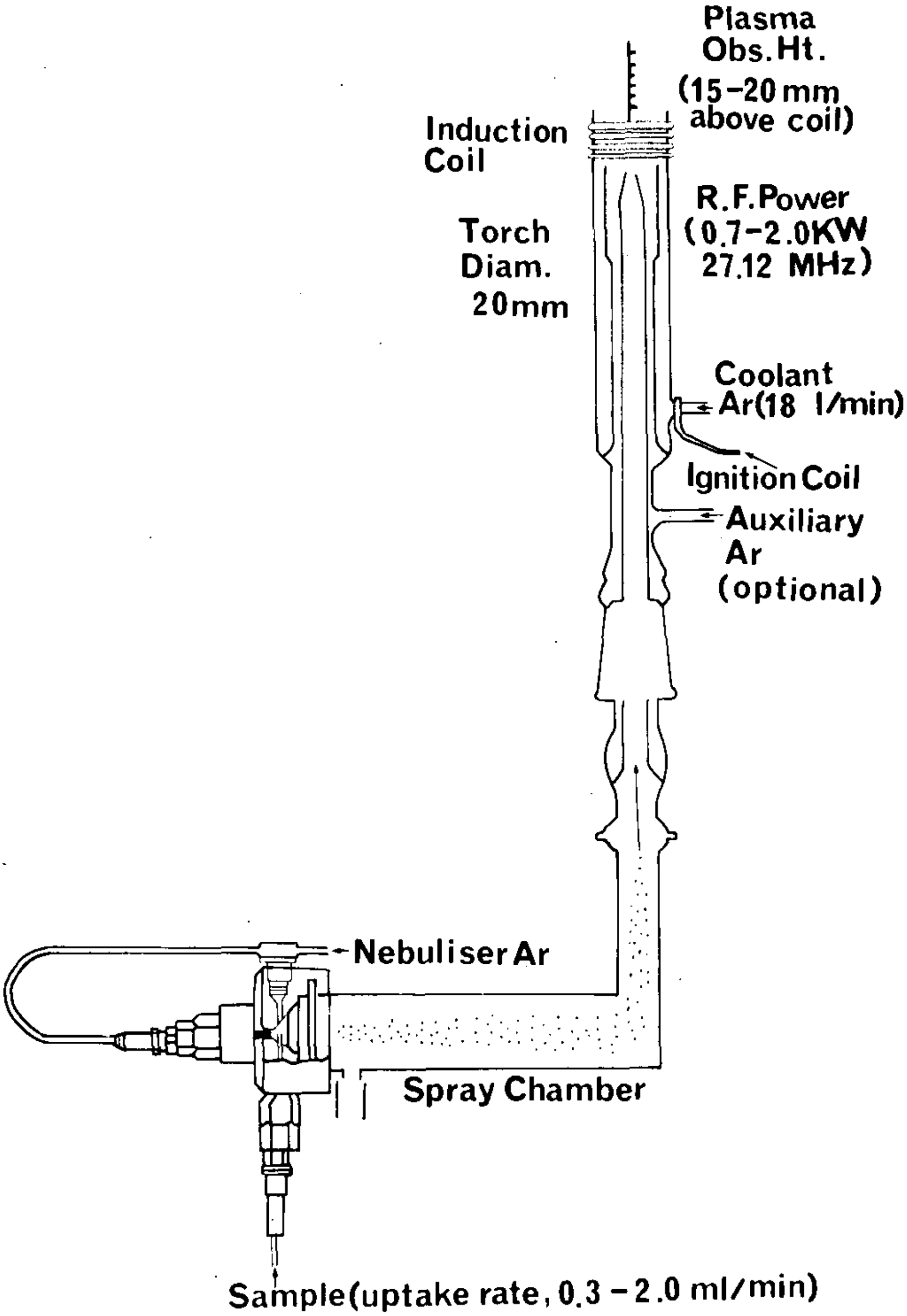

Fig. 1. ICP torch assembly and cross flow nebulizer.

are again controlled entirely from the teletype.

The SIT detector is one of a number of solid state multichannel devices that has been finding increasing use in spectroscopic measurement systems[5]. The detector is placed at the exit port of the monochromator (exit slit removed) and provides a simultaneous monitoring capability over a specific wavelength range which is determined primarily by the reciprocal linear dispersion of the monochromator. For the present system a 5 nm spectral window, subdivided into 500 electronic channels, is displayed across the tube face. The SIT consists of an array of light sensitive elements which develop a change pattern on exposure to uv/vis radiation. The change pattern is addressed by a scanning electron beam and through the optical multichannel analyser (OMA) control unit the signal can be displayed in real time on an oscilloscope or stored in memory for further processing, e.g. signal accumulation. The OMA unit has two separate memory areas (memory A and memory B) for signal storage so that plasma background-corrected spectra (A-B mode) can readily be afforded. Such emission spectra are of particular value for the study, classification and correction of plasma interference effects and it is in this respect that SIT detection offers certain advantages relative to PMT detection for plasma applications. The quantum efficiency of the SIT is relatively poor in the low uv but for analyte emission wavelengths greater than approximately 250 nm, detection limits for SIT and PMT detection are comparable[6].

For Pepperbush analysis both instruments are used to provide quantitative data but in addition for qualitative studies such as checking for potential plasma interference effects, the SIT instrument is employed. Further details regarding the performance characteristics of both instruments are indicated in later sections.

**Table I**

*Description of Instrumentation and Operating Conditions*

| | Jarrell-Ash Atomcomp | Purpose Built (Computer-controlled) |
|---|---|---|
| Spectrometer | Polychromator<br>Dispersion, 0.53 nm/mm in 1st order. | Monochromator (Jobin Yvon)<br>Dispersion, 0.4 nm/mm in 1st order. |
| Detection | PMT (Multichannel) | SIT (Single channel) |
| Generator | Plasma Therm | Shimadzu |
| Frequency (MHz) | 27 | 27 |
| Load Coil | 4 turns | 2 turns |
| Normal Operating Conditions | | |
| Forward Power (Kw) | 1.1 | 1.6 |
| Coolant (l/min) | 18 | 10 |
| Carrier (l/min) | 1 | 1 |
| Auxiliary (l/min) | 0 | 1.6 |
| Observation Height (mm) | 19 | 17 |
| Nebulizer and<br>Uptake Rate (ml/min) | Cross-flow type<br>~1.3 | Concentric type<br>~2.5 |

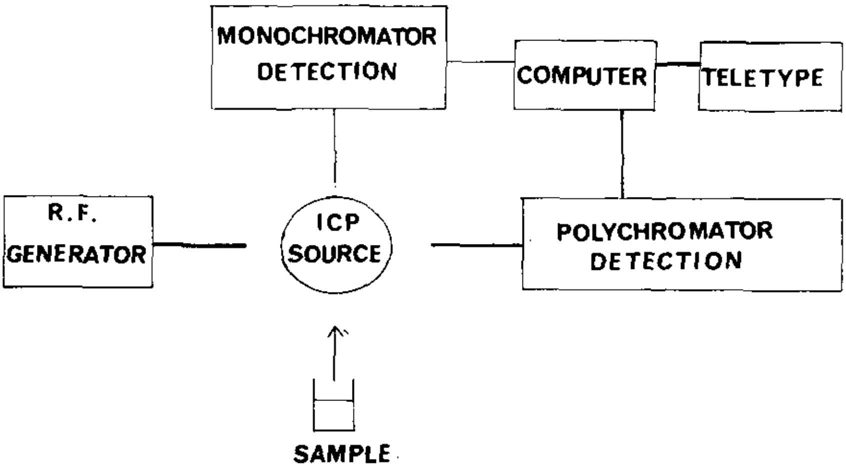

Fig. 2. Main components of direct reading ICP emission spectrometer.

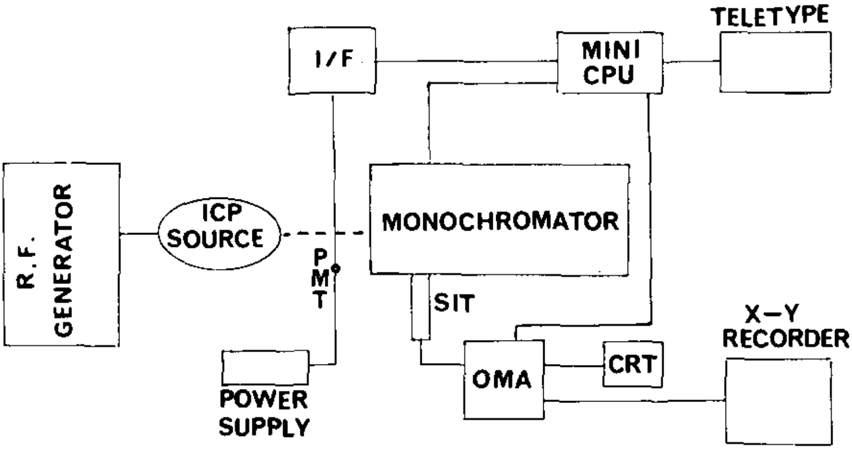

Fig. 3. Main components of SIT-based ICP emission spectrometer.

*Analytical Considerations*

## 1. Introductory Remarks

In this section, features and operational characteristics of the ICP technique are introduced and attention is drawn to various factors which may contribute to accuracy and precision of ICP measurement. Of concern are factors such as sample dissolution, standard solution composition and calibration, physical and spectral interference and instrument stability. At first, the principal plasma operating parameters are considered since they determine to a great extent analytical performance characteristics.

## 2. Principal Plasma Operating Conditions

For simultaneous multielement analysis (SMA) a basic requirement for all techniques is the selection of a single set of operating parameters; in many cases compromises are made between sensitivity, interference and applicability. Fortunately in plasma emission spectrometry a single set of operating conditions can be found to give high sensitivity for many elements and this contrasts with other techniques such as flame spectrometry where optimum conditions for elements are very dependent on flame conditions[4,7].

In ICP emission spectrometry 3 plasma operating parameters, namely radiofrequency (RF) power, carrier gas flow rate and the plasma observation zone, have been found to play a decisive role in determining detection performance for a particular element[8]. To obtain the optimum plasma operating conditions for a particular element one approach is to maximize the signal to background ratio (S/B) for the element, it being assumed that the maximum S/B corresponds to the maximum signal to noise ratio (S/N) and hence the lowest detection limit. This approach is valid for those systems whose noise is source-dominated and where the standard deviation of the background noise is directly proportional to the background level[9,10,11]. Before the results for the S/B optimization studies are presented, some basic comments concerning the role of the principal operating parameters are necessary. First let us consider the uv/vis spectrum of the plasma which is shown in Fig. 4. The spectrum is complex with many Ar lines superimposed on the continuum background and spectral features characteristic of species such as H, OH, NH, NO, C and CN can be identified. The region from 200 - 350 nm is particularly suitable for analysis since plasma background is relatively low and also because intense atomic and ionic emission lines of elements are located in this region (see Table II).

At positions near to the load coil (observation region approx. 0 - 10 mm above the coil), the plasma temperature is high (7000 - 8000 K) and the argon continuum background is intense. This region is not usually favourable for observing analyte emission on account not only of the high background but also because the relatively short analyte residence time is insufficient to vaporize and excite the analyte. On moving up the plasma the temperature gradually decreases and observation zones high in the plasma, e.g. 30 - 60 mm are also not usually favourable where temperatures approaching that of chemical combustion flames are obtained. An intermediate region, 15 - 20 mm, which corresponds to analyte residence times of the millisecond order is favourable for analytical measurement.

Increase of the RF energy results in a rise in plasma temperature and a corresponding rise in the plasma background (the plasma volume and luminosity are also increased). Although for most elements maximum emission intensity is favoured by a high temperature, the magnitude of the background intensity level determines to a great extent the optimum

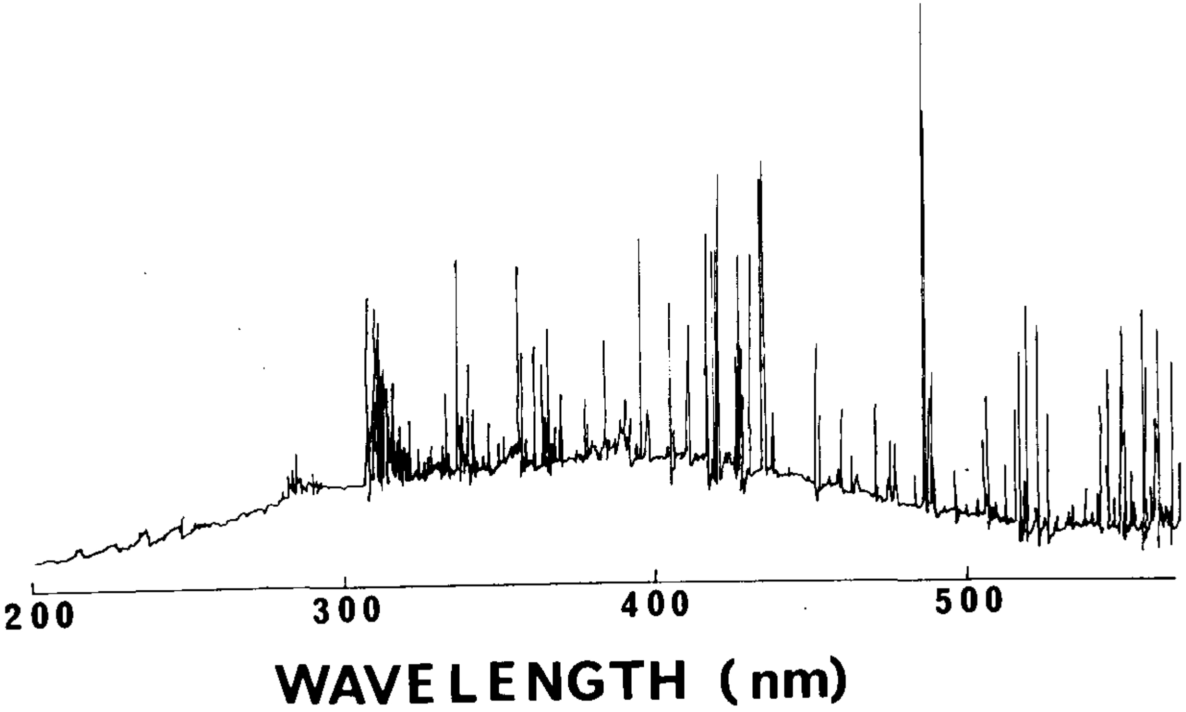

Fig. 4. UV-VIS spectrum of argon ICP
plasma operating conditions, R.F. power 1.5 kw; observation height, 17 mm; sample uptake rate~1.0 ml/min.

power level.

The 3rd important variable, the argon carrier gas flow rate, serves to nebulize the sample and to transport the aerosol droplets to the plasma. For sufficiently high carrier gas flow rates, the sample penetrates the plasma and travels up the narrow central axial channel. At very high flow rates performance is, however, impaired due to cooling of the plasma and also

**Table II**

Optimum Values of Principal ICP Operating Parameters and Detection Limits

| Element | Wavelength (nm) | Power (Kw) | Obser. Ht above coil (mm) | Sample Uptake Rate (ml/min) | D.L.* (ppm) |
|---|---|---|---|---|---|
| Ag | 328.1 | 1.1 | 24 | 1.1 | 0.001 |
| Al | 308.2 | 1.1 | 20-22 | 1.0-1.1 | 0.017 |
| As | 193.7 | 0.9 | 18-20 | 0.9-1.0 | 0.020 |
| B | 249.8 | 0.9-1.0 | 19-21 | 1.1 | 0.001 |
| Be | 313.0 (II) | 1.7 | 16-18 | 0.95 | 0.0008 |
| Ca | 317.9 (II) | 1.1-1.2 | 19 | 1.0-1.1 | 0.005 |
| Cd | 228.2** | 1.0-1.1 | 18-20 | 0.9-1.0 | 0.001 |
| Co | 228.6 (II) | 1.1 | 18-20 | 0.9-1.0 | 0.002 |
| Cr | 267.7 (II) | 1.2-1.3 | 20 | 1.0 | 0.005 |
| Cu | 324.8 | 0.95 | 22 | 1.1 | 0.0008 |
| Fe | 259.9 (II) | 0.9-1.0 | 23 | 1.2 | 0.002 |
| [1]Hg | 253.6 | 1.1 | 16-18 | 1.1 | 0.014 |
| [1]K | 766.5 | 0.85 | 12 | > 1.6 | 0.008 |
| Mg | 279.6 (II) | 1.1 | 17 | 0.95 | 0.00007 |
| Mn | 257.6 (II) | 1.6 | 17-18 | 1.0 | 0.0006 |
| Mo | 202.0 (II) | 1.3 | 18-19 | 1.0-1.1 | 0.005 |
| [1]Na | 589.0 | 0.8-0.9 | 14-15 | 1.1-1.4 | 0.0008 |
| Ni | 231.6** (II) | 0.85 | 18 | 0.9-1.1 | 0.006 |
| P | 214.9** | 1.2 | 16-18 | 1.2 | 0.04 |
| Pb | 220.4 (II) | 0.9-0.95 | 19 | 1.1 | 0.011 |
| Sb | 217.6 | 0.9-1.0 | 19-20 | 1.1 | 0.006 |
| Se | 196.0 | 1.0 | 17-18 | 1.0-1.1 | 0.022 |
| Si | 288.2 | 1.1 | 21 | 1.0-1.1 | 0.010 |
| Sn | 189.9 (II) | 1.1-1.2 | 18 | 0.85-0.95 | 0.021 |
| Ti | 334.9 (II) | 1.0 | 19-20 | 0.95 | 0.0008 |
| V | 292.4 (II) | 1.1 | 20 | 1.1 | 0.003 |
| Zn | 213.9 | 1.1 | 16-18 | 0.9 | 0.002 |

* Plasma operating conditions: power, 1.1 Kw; observation height, 19 mm; flow rate, 1.0 ml/min.

(II) refers to ionic line.

** refers to 2nd order.

1 data were obtained by monochromator detection.

because of a reduced analyte residence time. Poor analytical performance is also obtained at low carrier gas flow rates where the aerosol droplet velocity is not sufficient to penetrate the plasma and instead the particles pass through the outer regions of the plasma2). It should be mentioned that nebulization efficiency is about 5%.

To determine the optimum operating conditions for simultaneous multielement determinations the maximum S/B for elements programmed into the direct reader was obtained. Experimentally, this involved location of the maximum S/B for the individual elements when one plasma parameter was varied while the other two were fixed (e.g. stepwise increments of power for fixed observation height and sample uptake rate). In this way, it was possible to obtain a set of plasma operating conditions which yielded the maximum S/B for the each element. Graphical presentation of the signal (S) and background (B) behaviour for 4 elements (Na, Cu, Zn, Ca) as a function of the plasma operating conditions is given in Fig. 5 (1, 2, 3). The elements Na, Cu, Zn and Ca were chosen for illustration since their emission behaviour was considered to encompass that of the elements investigated in this study (see Table II).

For the power variation study (Fig. 5.1), element emission intensity increases with increasing power (not continuously up to 2.0 kw for the atom lines of Na and Cu where levelling-off occurs) and maxima in the respective S curves do not occur. The accompanying increase of plasma background (with power) at all wavelengths counteracts the emission increase and the optimum S/B tends to occur at low power levels for the 4 elements ( 1.1 kw). It should be noted that the behaviour of the Zn atomic line is similar to that of the Ca ionic line and not to the Cu atomic line, definite maxima occurring for the S/B curves of the former two elements. This is in agreement with the findings of Berman and McLaren8) who considered that the relatively high excitation energy of the Zn atom line (5.8 eV cf. 3.8 eV for Cu) is responsible for emission behaviour similar to that of ionic lines. The behaviour of the other elements investigated (see Table II) utilizing either ionic or atomic emission was similar to that of Ca.

In explaining the emission dependence on plasma observation height (see Fig. 5.2), the important factors to consider are (1) the progressive decrease in plasma temperature with increasing observation height and (2) the analyte residence time appropriate to the measuring position. Na (also K) being an easily ionized element favours a low observation height corresponding to a short plasma residence time to provide the maximum atomic emission. Higher observation heights are necessary in the case of Cu, Zn, Ca to ensure complete vaporization/excitation and in contrast to that for Na well-pronounced maxima centred around the 15 mm region were obtained from the respective S curves. The continuum plasma background decreases with increasing observation height so that the maximum in the S/B curves are shifted to slightly higher heights relative to that for the S curves. Zn is an exception where because of the presence of NO, the background commences to increase with increasing observation height beyond about 17 mm. That is, the NO population increases progressively with observation height beyond approximately 17 mm due to air entrainment and consequently the background emission at 213.9 nm also increases. This effect is responsible for the coincidence of the Zn S and S/B curves (cf. Cu, Ca).

Indefinite increase of the sample uptake rate although causing a reduction in background intensity due to cooling of the plasma also results in a decrease in emission intensity. Peaking of the individual S and S/B curves (not coincident) occurred for Zn, Ca and Cu in contrast to that for Na. It can be seen (Fig. 5.3) that a relatively high sample uptake rate is favourable for atomic emission of Cu and Na (particularly the latter) while in the case of Ca(II) and Zn(I), the optimum sample uptake rate is about 1.0 ml/min.

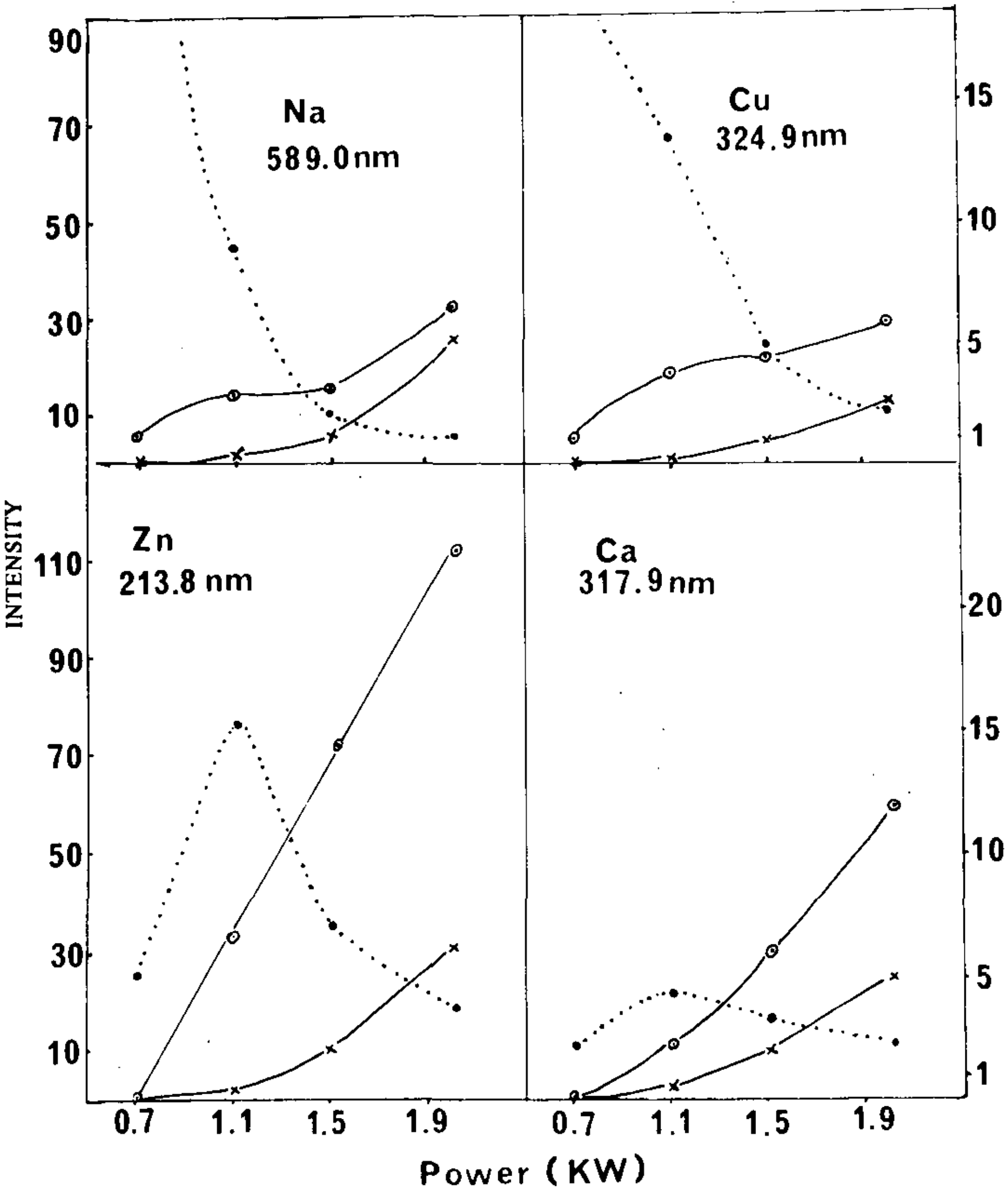

Fig. 5.1. Signal (S), background (B) and S/B for selected elements as a function of power. Element concentrations, 1 $\mu$g/ml; observation height, 19 mm; sample uptake rate, ~1.0 ml/min. ○——○ , S ; ×——× , B ; •·········• , S/B.

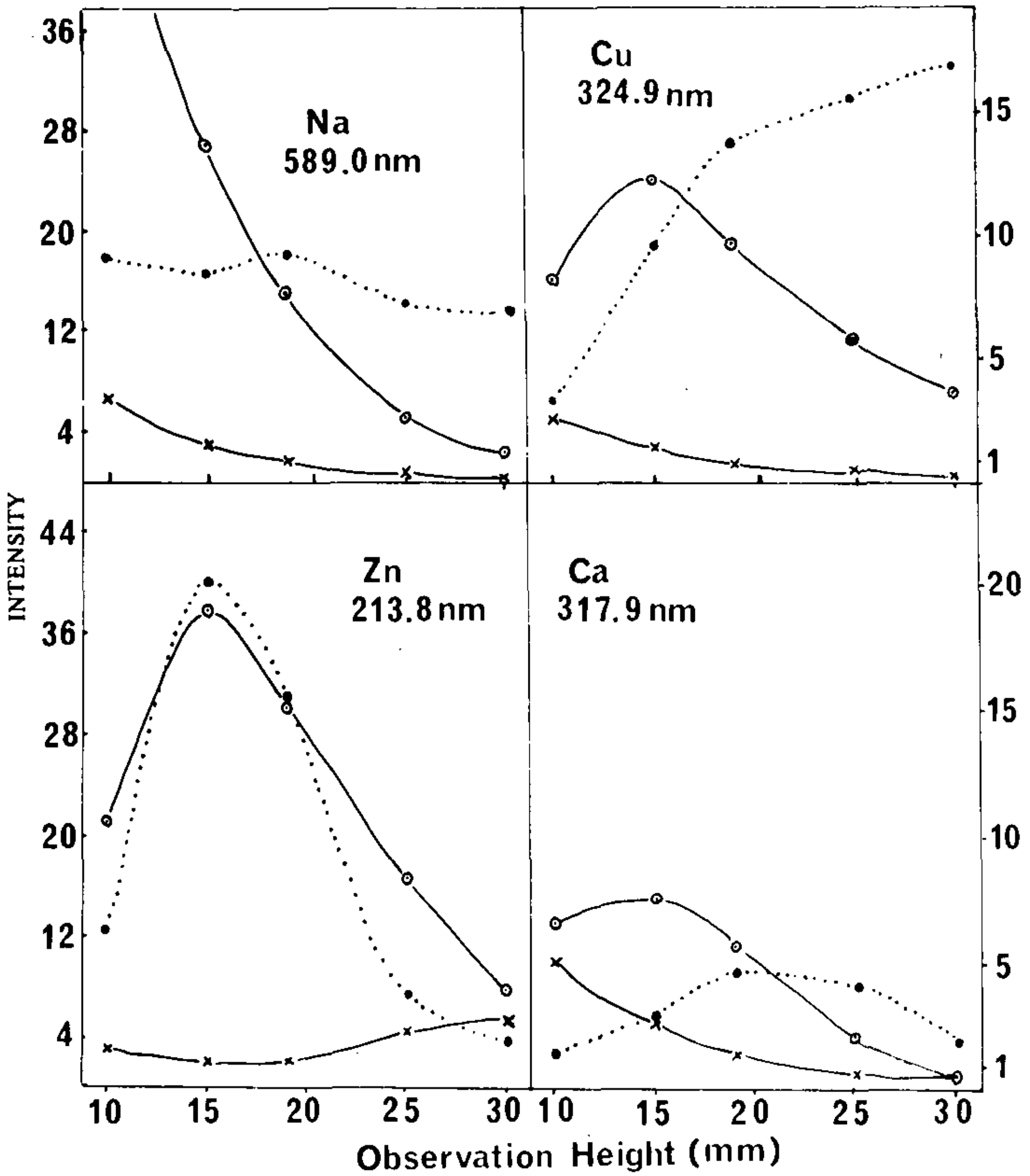

Fig. 5.2. Signal (S), background (B), and S/B for selected elements as a function of observation height.
Element concentrations, 1 $\mu$g/ml; power, 1.1 kw; sample uptake rate, ~1.0 ml/min.
○——○ , S; ×——× , B; •·········• , S/B.

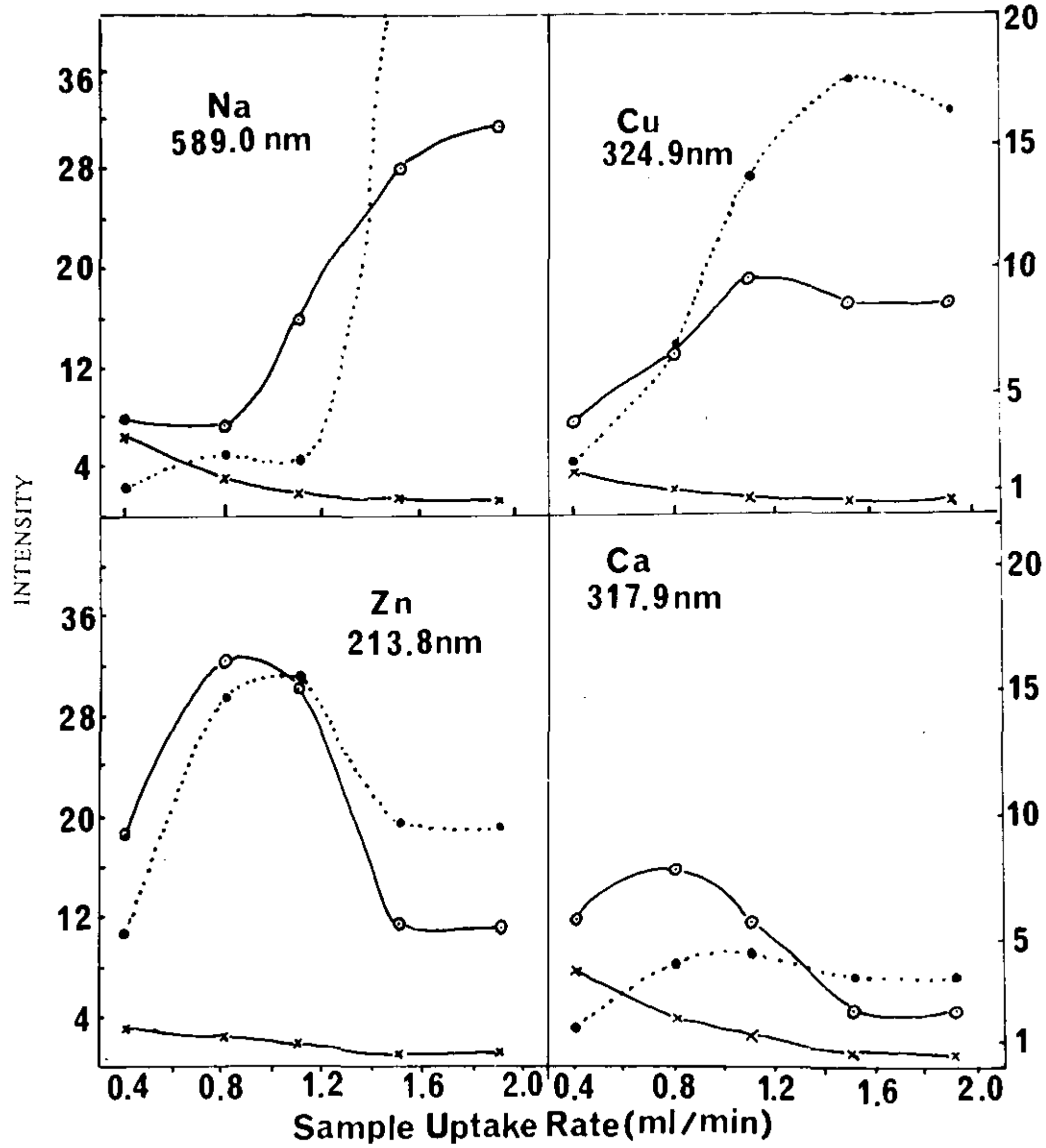

Fig. 5.3. Signal (S), background (B), and S/B for selected elements as a function of sample uptake rate.
Element concentrations, 1 μg/ml. power, 1.1 kw; observation height, 19 mm.
o——o ,S; x——x ,B; •·······• ,S/B.

The optimum values of the plasma operating parameters for the individual elements are presented in Table II. Clearly a significant feature of the data is that the spread in the individual values for the elements is quite narrow, but atypical behaviour as indicated in the S/B curves can be noted for the easily ionized alkali metals, Na and K, which favour a relatively low power and high sample uptake rate. In the last column of Table II, detection limit data for the elements at fixed operating conditions, namely RF power 1.1 kw, observation height 19 mm, and sample uptake rate 1.0 ml/min, are included. These results indicate that a simultaneous detection capability at ppb levels is realized for a single set of plasma operating conditions.

### 3. Sample Dissolution

The ICP technique invariably requires sample to be in liquid form so that for solid sample types a preliminary destruction of the organic matter either by wet or dry ashing is required. ICP nebulization efficiency is sensitive to the final acid and dissolved solids content so that for both digestion procedures careful control of the dissolution process is required.

For wet digestion, procedures based on the method of Feldman12) and using mixtures of nitric, perchloric and sulphuric acids are suitable. Dahlquist3), for example used a 1:1 mixture of nitric and perchloric acid for the digestion of botanical sample (25 ml for 1 g sample). The final dilution factor should be chosen after consideration of the element concentrations in the digested solution and the respective ICP detection limits. Frequently it is not possible to achieve simultaneous multielement determinations of trace, minor and major elements with a single dilution step. For instance Ca, Mg and K are usually at high concentrations in botanical samples so that further dilution of the digest may be required to bring the signals within the dynamic range of the instrument13). Insolubility of perchlorates3,13) may prevent the realization of low dilution factors; for an initial 1 g sample the final digest volume may be limited to between 10 and 50 ml (depending on sample type and digestion procedure).

Dry-ashing3,13,14) has also been adopted for ICP analysis of botanicals, the principal advantage over wet digestion being that there are not solubility limitations and a practical sample dilution factor of 1 g per 5 or 10 ml of aqueous solution is possible13). The major limitation of the technique, however, is the loss of volatile elements such as Hg, Cd, As and Se during ashing.

### 4. Standard Solutions and Calibration

In the preparation of multielement standard solutions for ICP analyses, a number of guidelines can be given. First it is imperative to use high purity metals or salts and acids and to check for reagent impurities in either. A list of high purity chemicals for stock solution preparation has been compiled by Ward15). The number of standard solutions used for calibration and the combination of elements depends on a number of a factors including solution compatability and stability, analysis requirements, and instrument and software capability. For the former, use of HCl and $H_2SO_4$ and corresponding salts should be avoided, if possible, since a number of elements, e.g. Ag, Ba and Pb, form insoluble salts13). Further it may be necessary to match the acid concentration of the standard solutions to that of the sample in order to eliminate differences in sample transport efficiency and/or plasma excitation mechanisms for the two solutions. It is also recommended that regular checking of standard solution stability is carried out, particularly for solutions containing elements at ppm concentration levels or less.

For multielement solutions the possibility of spectral interference effects should be considered (see section 5). If interferences are found to be significant and cannot be avoided such effects should be quantified and corrected for in the calibration scheme.

ICP calibration curves are essentially linear over 4-5 orders of magnitude so that in most applications a 2-point standardization procedure can be adopted where the concentrations of the standards, denoted the high and low standards, bracket that of the unknown; in practice distilled water or the appropriate blank solution can serve as the low standard (zero concentration). It is the responsibility of the analyst, however, to check that the calibration scheme adopted is appropriate for the particular element(s) and sample type.

The magnitude of instrument measuring precision should be evaluated. Instrumental drift primarily related to sample introduction and nebulizer operation may be a limiting factor and normally long-term (hours) instrument stability is poorer than short-term (minutes) stability. In practice, frequent calibration of the instrument minimizes the effects of drift.

## 5. Matrix Effects and Correction

Matrix interference effects in ICP emission spectrometry are small compared to those for other instrumental techniques such as a d.c. arc and spark emission spectrometry, atomic absorption spectrometry, X-ray fluorescence spectrometry and neutron activation analysis. In the analysis of botanical samples, however, certain types of interference primarily associated with the major matrix components may occur and as a result analytical accuracy and precision are degraded. First, it is important to characterize and quantify the effects and for this it is necessary to distinguish between the physical interference effects, i.e., those related to nebulization and sample introduction, and spectral interference effects which originate in the plasma.

In the case of plasma interference effects the possibility of spectral line interference and instrumental stray light effects associated with the alkaline earth elements Ca and Mg are of concern and have been well documented2,3). Chemical and ionization interferences, a feature of atomic absorption spectrometry, are unimportant2). Spectral line interference which results when non-analyte emission reaches the detector is common to all emission techniques (rare in atomic absorption spectrometry) and flexibility in wavelength selection is a desirable feature in plasma instrumentation. When spectral line interference is unavoidable, quantification of the interference and correction (usually computer-assisted) may provide valid data. This lack of specificity for the plasma technique is an undesirable feature but generally for botanical samples the potential for direct spectral line interference is low relative to other matrix types such as steels and geological samples.

The consequence of instrumental stray light is a general rise in the plasma background which in the analysis situation corresponds to a positive bias in the result. The level of stray light is dictated primarily by the stray-light rejection qualities of the instrument and various approaches can be used to further reduce or eliminate stray light16,17). Such interference effects are generally significant only when determinations are being performed near the detection limit for the particular element2). Initially sample type should be assessed to determine the Ca and Mg content. If stray light does cause significant error then background correction and/or matrix duplication of the standard solutions would be required. The use of the SIT system for correction of stray light either manually or under computer-control has been described18).

## 6. Analytical Method

The basic feature of the ICP procedure adopted is that major, minor and trace elements of interest were determined simultaneously for a single dilution of the sample digests. The two point calibration scheme further simplified analytical operations. To allow judgement on the precision and accuracy of the total analytical procedure Orchard Leaves SRM was analyzed in an identical manner to Pepperbush.

### 1) Sample Dissolution

Pepperbush (2 g, in triplicate) and Orchard Leaves (2 g, in triplicate) were digested in teflon beakers on a hot plate using a mixture of nitric and perchloric acid. At the end of digestion excess acid was removed and the digests were quantitatively transferred to volumetric flasks (200 ml) and diluted to the mark with distilled water. Solutions were then filtered (precleaned millipore, 0.45$\mu$) and transferred to precleaned polyethylene containers. Blank solutions (quadruplicate) for the total procedure were also obtained. The dissolution process is described in detail in the chapter on atomic absorption analysis.

The dissolution factor of 2 g/200 ml was decided upon mainly after considering the element concentration levels in the Pepperbush digests and the upper and lower determination limits for the ICP calibration curves. To eliminate possible "acid effects" in ICP measurement, the dissolution procedure was such that a final acid content of approximately 0.1 M perchloric acid was achieved.

### 2) Standard Solutions

For the elemental analysis of Pepperbush and Orchard Leaves digests, a single multielement solution in 0.1 M perchloric acid served as the high standard while for the low standard distilled water was used. The concentrations for the individual elements are indicated in Table III and were selected after considering preliminary ICP data for Pepperbush. A multielement solution identical to the above but not containing K (150$\mu$g/ml), Ca (150 $\mu$ g/ml) and Mg (50 $\mu$ g/ml), was prepared in order to determine whether the major matrix components influenced data. Single-element stock-solutions (1000 $\mu$g/ml) which originated from either high purity metals or salts were used for the preparation of the multielemental standard solutions. Please consult the corresponding discussion in the atomic absorption chapter for further details.

### 3) Instrument Operation

#### i) Direct Reader

All analytical results were obtained under normal plasma operating conditions, i.e. RF power 1.1 kw, observation height 19 mm, sample uptake rate approx. 1.3 ml/min. Potential errors due to instrumental drift were minimized by adopting an analysis sequence whereby sample solutions were analyzed immediately after standardization. The analysis cycle which was controlled by teletype required about 4 minutes and generated two results for each element. The cycle consisted of (1) aspirating alternately the low and high standard solutions (in that order) and recording of the multielement signal intensities (two separate 10 s integrations), (2) standardization of the instrument (storage of the calibration curves in computer memory) and (3) aspirating and recording of the multielement signal intensities of the sample solution (in an identical manner to the high and low standards) and finally output of the data in concentration units. The above cycle was performed 4 times for each

Table III
Composition of Standard Solutions

| Element | High Standard | Low Standard |
|---|---|---|
| Ca | 150 | 0 |
| K | 150 | 0 |
| Mg | 50 | 0 |
| Mn | 20 | 0 |
| P | 10 | 0 |
| Al | 5 | 0 |
| Zn | 5 | 0 |
| Fe | 5 | 0 |
| Pb | 5 | 0 |
| Cd | 1 | 0 |
| Co | 1 | 0 |
| Cr | 1 | 0 |
| Cu | 1 | 0 |
| Ni | 1 | 0 |

Units $\mu$g/ml.
High Standard, 0.1 M $HClO_4$.
Low Standard, distilled water.

sample solution and thus 8 analytical results for each element were obtained. Further, the above analysis procedure was carried out on three separate days for each sample type so that a data base of 72 results for each element was achieved (24 results per sample).

For those elements (Sr, Ba, Na, K) not programmed into the direct reader single element determinations ($n \geqq 15$; on one day) were performed using monochromator detection.

ii) SIT system

Normal plasma operating conditions were employed for analysis, i.e. R.F. power 1.6 kw, observation height 17 mm and sample uptake rate~2.5 ml/min. Analytical wavelengths were addressed sequentially and thus the extended analysis time (relative to simultaneous multielement analysis) limited the number of replicate analyses to one. The SIT-OMA detection system utilized an accumulation time of 4.1 s which corresponded to 125 machine scanning cycles for the integration time of 32.8 ms. The standardization procedure consisted of aspirating alternately the standard and blank solutions and storing the respective emission spectra in the A and B memory areas of the OMA. The subtracted spectrum (A-B) provided the channel-by-channel difference (5 nm displayed over the 500 electronic channels) and the intensity of emission lines, derived after subtraction of side background, were employed for generating calibration curves. The unknown solution and blank were next processed in an identical manner and concentrations were derived by referring the intensities of emission lines (corrected) to the calibration curves stored in computer memory. A computer print-out for the standardization and analysis procedure is given in Section 5 in the case of Mn.

### *Results and Discussion*

The analytical values for Pepperbush and Orchard Leaves as determined by ICP emission spectrometry are presented in Table IV. Values for element content are expressed as the mean of n independent determinations. (Direct reader: Pepperbush, n=72; Orchard Leaves, n=48. SIT system: $n \geq 5$). The uncertainty limits were computed from the statistic, t x standard deviation (estimate), for the 95% confidence level. It should be appreciated that the determination of major, minor and trace elements (for both instruments) was accomplished with a single dilution of the sample digests, and, further, for standardization only a single multielement solution (high standard - see Table III) and distilled water (low standard) were employed.

Before judging the accuracy and precision of the reported values it is informative to consider the elemental content for the two botanical reference materials. Dr. Okamoto has already discussed in Chapter II the metal accumulation properties of Pepperbush and the ICP data of Table IV confirm this; order of magnitude concentration differences are apparent for Cd, Co, Mn, Ni and Zn. Only in the case of Pb is the concentration level in Pepperbush substantially lower than in Orchard Leaves. The remainder of the elements including the major elements Ca and K occur at similar concentrations in both materials.

To illustrate differences in elemental content, plasma emission spectra (A-B mode) for digests of Pepperbush and Orchard Leaves are presented in Figs. 6 and 7. The background correction facility of the SIT-OMA system was utilized to remove plasma background emission and, thus, the spectra obtained are comparatively simple consisting only of analyte emission. The wavelength region of 256 nm to 261 nm contains the emission triplet of Mn (257.6 nm, 259.4 nm and 260.6 nm) and in Fig. 6 differences in the Mn content of Orchard Leaves and Pepperbush are striking. In Table V, the computer print-out for standardization and analysis of Pepperbush utilizing the Mn triplet is presented. Channel numbers 411, 227, and 101 correspond to the Mn emission wavelengths of 257.6 nm, 259.4 nm and 260.6 nm, respectively. It can be noted that the Mn content of the Pepperbush digest is essentially identical to that of the multielement standard solution ($20\mu$g/ml).

In Fig. 7, emission lines of a number of elements are contained within the spectral region 212 nm to 216 nm and again intensity differences are apparent for the elements (e.g. Zn, P, Cd). A further interesting feature to note is that the baseline of the spectra do not coincide with the zero intensity level. This rise in the plasma background level, a result of stray light reaching the detector (particularly the intense Ca 393.4 nm emission) has been discussed in a previous section.

Accuracy and precision of the ICP procedure are now assessed by comparing the analytical data for Orchard Leaves with the certified values. It can be seen from Table IV that agreement with the certified values is fairly satisfactory but for certain elements, e.g. Ca and P, accuracy has not been attained, i.e. the analyzed values (mean $\pm t\sigma$) do not lie within the bounds of the certified values. The inaccuracy for Ca was traced to an inappropriate calibration scheme; the Ca concentration of the Orchard Leaves digest was elevated relative to that of the standard solution (208 $\mu$g/ml *vs* 150 $\mu$g/ml) and non-linearity of the calibration curve was found to impart a negative bias on the Orchard Leaves' result. Dilution of the Orchard Leaves' solution (1/100) and analysis using a 2 $\mu$g/ml Ca standard yielded the accurate value of 2.08±0.03%. The method blank was significant for Cu and Na and blank correction was performed.

A number of limitations of the ICP technique are apparent from inspection of Table IV. Lack of sensitivity prevented detection of Cd and Co in Orchard Leaves and in the case of

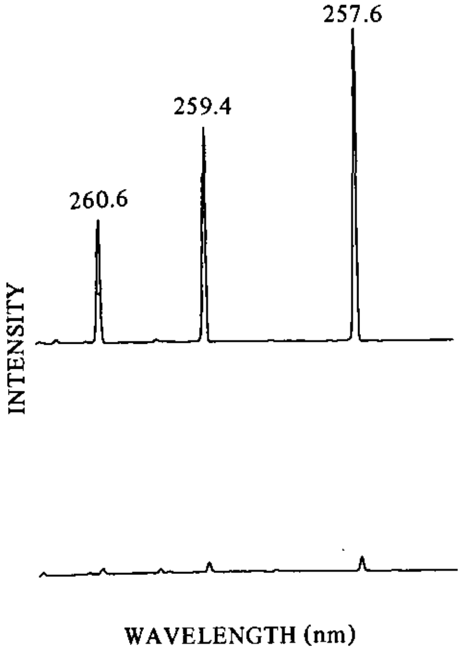

Fig. 6.
SIT emission spectra (A-B mode). Mn triplet for Pepperbush (upper) and Orchard Leaves (lower) digests. Dissolved solid content approx. 1 g per 100 ml. Plasma operating conditions, see text.

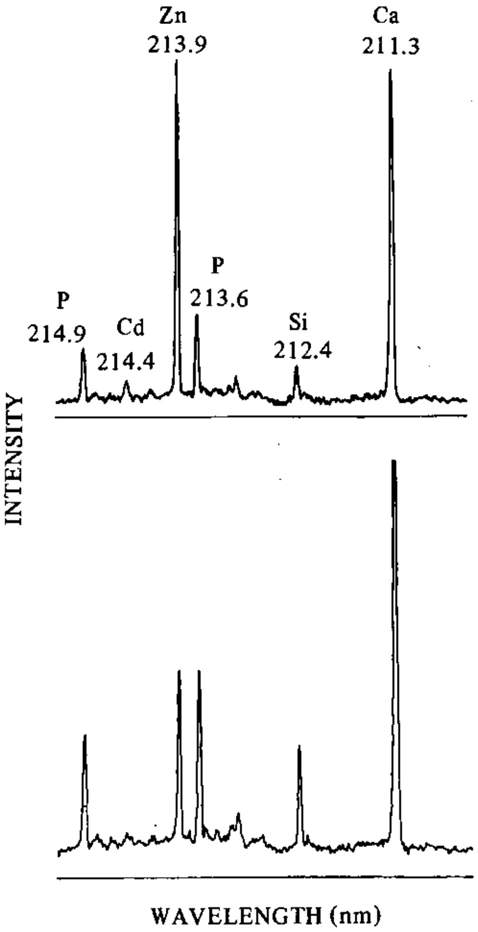

Fig. 7.
SIT emission spectra (A-B mode) for Pepperbush (upper) and Orchard Leaves (lower) digests. Dissolved solid content approx. 1 g per 100 ml. Plasma operating conditions, see text.

**Table IV**
Analytical Values for Pepperbush and Orchard Leaves
(Mean ± 95% Confidence Level)

| Element | Pepperbush |  | Orchard Leaves |  |
|---|---|---|---|---|
|  | Direct Reader | SIT | Direct Reader | Certificate |
| Al | 431±30 | - | 356±22 | - |
| Ba | 163±8 | 173±5 | 39.5±2.8 | - |
| *Ca | 1.44 ±0.07 | 1.49±0.07 | 1.87±0.07 | 2.09±0.03 |
| Cd | 7.3±0.4 | 7.7±2 | ND | 0.11±0.02 |
| Co | 21.4±0.8 | 20±3 | ND | (0.2) |
| Cu | 11.3±0.9 | - | 12.2±0.8 | 12±1 |
| Fe | 204±6 | 217±7 | 286±49 | 300±20 |
| *K | 1.49±0.23 | - | 1.41±0.19 | 1.47±0.03 |
| Mg | 4264±160 | 4130±111 | 5868±240 | 6200±200 |
| Mn | 2086±64 | 2130±45 | 84.9±2.6 | 91±4 |
| Na | 107±20 | - | 92±8 | 82±6 |
| Ni | 8.4±1.2 | - | 1.8±1.4 | 1.3±0.2 |
| P | 1031±38 | 971±164 | 1884±48 | 2100±100 |
| Pb | ND | - | 44±5 | 45±3 |
| Sr | 34.2±0.5 | 34±2 | 33.5±2.4 | (37) |
| Zn | 339±12 | 333±9 | 26.3±1.3 | 25±3 |

Units μ g/g except *wt %.
ND, not detected.

Cr, Ni and Pb, determinations were close to the determination limits and results were imprecise. Improvement of method sensitivity would be realized by adopting lower dilution factors for the digest but difficulties connected with solubility and nebulization may occur. Data for Na and K were also unreliable as indicated by the magnitude of the uncertainty limits. As already discussed in Section 3 the high temperature environment of the plasma is inappropriate for the easily ionized alkali metals. The analysis situation would be improved by selecting more favorable plasma operating conditions but this contradicts with the SMA requirement.

Digestion of both reference materials in triplicate and analysis over a 3-day period (Orchard Leaves, 2 days) provided information on method precision. Variance analysis revealed that the variability contribution from sample processing was of similar magnitude to instrument measuring precision and further the between - day contribution was negligible. An exception to the former concerned the Fe result for Orchard Leaves where relatively poor precision (cf. Pepperbush) resulted from variability in sample processing (assuming sample homogeniety). The analytical values for the three independent analyses were 319 μg/ml, 272 μ g/ml and 269 μ g/ml. A similar situation occurred for analysis of Ba in Pepperbush, the respective values being 140 μ g/ml, 147 μ g/ml and 54μ g/ml; the latter value was rejected.

Instrument measuring precision for the SIT system was poorer than for the direct but a strict comparison is not possible due to major differences in equipment and plasma operating conditions. It should be noted that the uncertainty limits for SIT data were computed from the results of a single Pepperbush digest and thus the data do not include the contribution from sample variability.

For an assessment of the Pepperbush data it is important to consider two points. First, the ICP technique does not suffer to an appreciable extent from interelement matrix effects and thus accuracy should not be degraded as a result of compositional differences between the two SRM's. In support of this, it was found that the absence of the principal matrix elements K, Ca and Mg from the high standard multielement solution (see Table III) did not influence the analytical results for Pepperbush. In addition since the composition of the high standard multielement solution was closely matched to that of the Pepperbush digest, and not Orchard Leaves, data for Pepperbush may be more reliable but particularly so for Ca. Further evidence for the reliability of the Pepperbush values is testified by the good agreement in results for the direct reader and the SIT system. SIT detection over the 5 nm wavelength range, in addition to providing a limited simultaneous multielement capability (see Fig. 7) enabled alternative emission lines of the same element to be utilized for simultaneous analysis, e.g. Mn (257.6 nm, 259.4 nm, 260.6 nm) and also Ca (317.9 nm, 315.9 nm), Mg (279.5 nm, 280.3 nm). This latter approach was valuable for confirming the absence of spectral interference.

For those elements determined but where certified values (for Orchard Leaves) are not available, e.g. Al, Ba, Co, Cr and Sr, an indication of reliability cannot be given. The chapter on certification should be consulted for an independent assessment of the accuracy of the Pepperbush data by ICP emission spectrometry.

**Table V**

Computer Print-out for Standardization and Analysis:
Determination of Mn in Pepperbush

```
NAME(MN   ) WAVE(2591.00) LEVEL( 2000) SIDE-BAND(3) MODE(2,2)

STANDARD SAMPLE    (.200E+02 PPM)

     CHANNEL PEAK(A) PEAK(B) PK(A-B) BACK GD  INTEN      A
     ------- ------- ------- ------- -------  -------  ----------
        101    30880    1856   29024     107   28917  .6916E-03
        227    38461    2888   35573      67   35506  .5633E-03
        410    61949    2653   59296      49   59247  .3376E-03

TEST SAMPLE

     CHANNEL PEAK(A) PEAK(B) PK(A-B) BACK GD  INTEN   CONCENT
     ------- ------- ------- ------- -------  -------  ---------
        101    31216    1855   29361     121   29240  .202E+02
        227    38777    2908   35869      75   35794  .202E+02
        410    62355    2655   59700      80   59619  .201E+02

OTHER PEAKS
```

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# CHAPTER VI

# ANALYSIS OF PEPPERBUSH BY ENERGY-DISPERSIVE X-RAY FLUORESCENCE SPECTROMETRY

T. Takamatsu

## *Introduction*

X-ray fluorescence spectrometry (XRF) has recently become one of the most attractive techniques for the determination of elements in environmental samples, especially in biological samples, on account of following reasons:

1. Sample preparation for XRF is considerably simple compared with many analytical techniques.
2. In the case of biological samples, matrix interference effects such as self-absorption and enchancement of X-ray radiation are comparatively small because the major constituents of biological samples are light elements such as C, H and N.
3. Detectability is fairly uniform across the periodic table and the technique is suitable for all elements from atomic number 11 (Na) upward.
4. XRF is a non-destructive technique so that the samples once analyzed can be retained.
5. The major and minor constituents can be analyzed simultaneously without resorting to dilution.
6. Spectral line interferences are relatively few because there are only a few X-ray lines for each element.
7. The XRF spectrum of an element, except that obtained with a high resolution spectrometer, is generally independent of the physical state and the chemical form of the element.

XRF offers two alternative measuring methods, wavelength-dispersion or energy-dispersion. The former has, in the past, predominated but recently the latter has also received considerable attention in many fields. Energy-dispersion type spectrometers have several advantages over wavelength-dispersion spectrometers:

1. The energy-dispersive technique offers a SMA (simultaneous multielement analysis) capability where the solid-state Si (Li) semiconductor detector, energy sensitive, allows the simultaneous detection of all X-ray lines without physical separation of the lines.
2. The Si (Li) detector is essentially 100% efficient over the X-ray energy range of general interest and the detector can be placed close to the sample. The sensitivity for each element, therefore, is much higher than that by wavelength-dispersive XRF and becomes a smoothly varying function of atomic number (Z).
3. A spectrum obtained by energy-dispersive XRF is amenable to computer reduction tech-

niques.

4. Energy dispersive X-ray fluorescence spectrometers also have advantages of low running cost, compactness and high stability because the mechanical construction is simple and only weak bombarding X-ray radiation is required.

NIES Pepperbush has been analyzed by energy-dispersive X-ray fluorescence spectrometry with an internal standard method.

*Experimental*

Instrumentation

An energy-dispersive X-ray fluorescence spectrometer (ORTEC, Model TEFA-6111) equipped with PDP-11/05 computer was used for elemental analysis of Pepperbush SRM. A spectrum analysis was performed with a SEEK program (developed by ORTEC) which includes background subtraction, peak searching, and Gaussian peak fitting. The measurement conditions of the X-ray fluorescence spectrometer are shown in Table I.

**Table I.**

X-ray Spectrometer Conditions

| | |
|---|---|
| Target: | Mo |
| Voltage: | 50 kV |
| Current: | 50 A |
| Filter: | Mo |
| X-ray path: | Air |
| Counting time: | 4000 - 10000 sec. |

Standards for Elemental Analysis

A series of artificial reference standards was prepared by adding known amounts of the desired elements to dried (80 ℃, 5 hrs) microcrystalline cellulose (Merk, for column chromatography) and by mixing it in an agate ball-mill for 1 hour. The cellulose powder appears to be most suitable as a base material for analysis of botanical samples. The concentration ranges of elements in the calibration standards were: 0-5% for K and Ca, 0-1000 ppm for Mn and Fe, 0-10 ppm for Ni and As, 0-25 ppm for Cu, 0-100 ppm for Zn, Rb and Sr, and 0-50 ppm for Pb. Appropriate amounts of the all elements, the range of which were mentioned above, were added to each calibration standard.

Sample Preparation

The standards and samples were dried in an oven at 85 ℃ for 4 hrs before analysis. Fifty $\mu$l of the internal standard solution (Cs: 100 mg/ml, Se: 1 mg/ml) were added to 500 mg of the artificial reference standards and to NBS standard reference materials (Orchard Leaves, Tomato Leaves, Spinach and Pine Needles). After drying again at 85 ℃ for 4 hrs and mixing in an agate ball-mill for 30 min., analysis was performed. The analytical

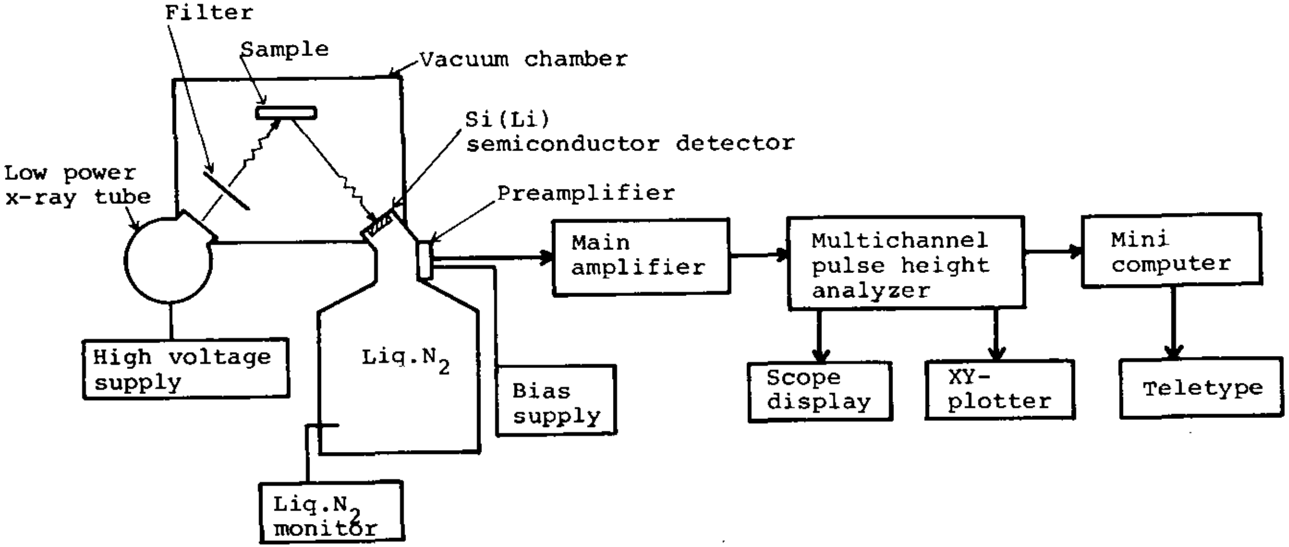


Fig. 1 Schematic diagram of energy-dispersive X-ray fluorescence spectrometer.

lines used were the L$\alpha_1$ for Pb and the K$\alpha_1$ for the other elements. The L$\alpha_1$ line of Cs was used as an internal standard for K, Ca, Ti, Mn and Fe, and the K$\alpha_1$ line of Se was chosen as an internal standard for Ni, Cu, Zn, As, Pb, Rb and Sr, as shown in Fig. 2. Internal standardisation is preferable in order to compensate for instrumental variations. The calibration curves were established by plotting the peak ratio, Ix/Ii.s., where Ix and Ii.s. are the peak intensities of the desired element and of the internal standard, respectively.

Some typical calibration curves are shown in Fig. 3. The calibration curves obtained with the artificial standards for Ni, Cu, Zn and Pb showed good linearity. Also, the calibration curves obtained with NBS botanical SRMs (plotting Ix/Ii.s. against the certified values for each element) were almost the same as those with the artificial standards. These results indicate that the XRF method used here is pretty good for elemental analysis of botanical samples. The X-ray spectrum of NIES Pepperbush is shown in Fig. 2 and the elemental contents were measured in the same manner using the calibration curves (Fig. 3).

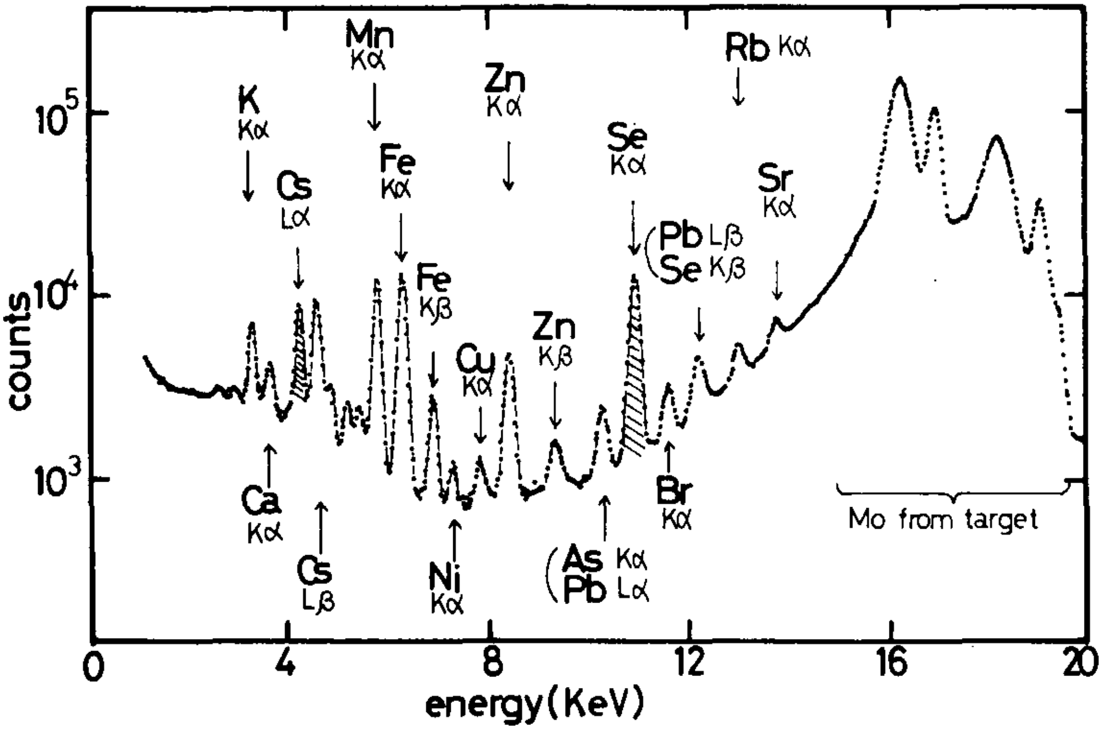


Fig. 2. X-ray spectrum of NIES Pepperbush
Shaded peak: Internal standard

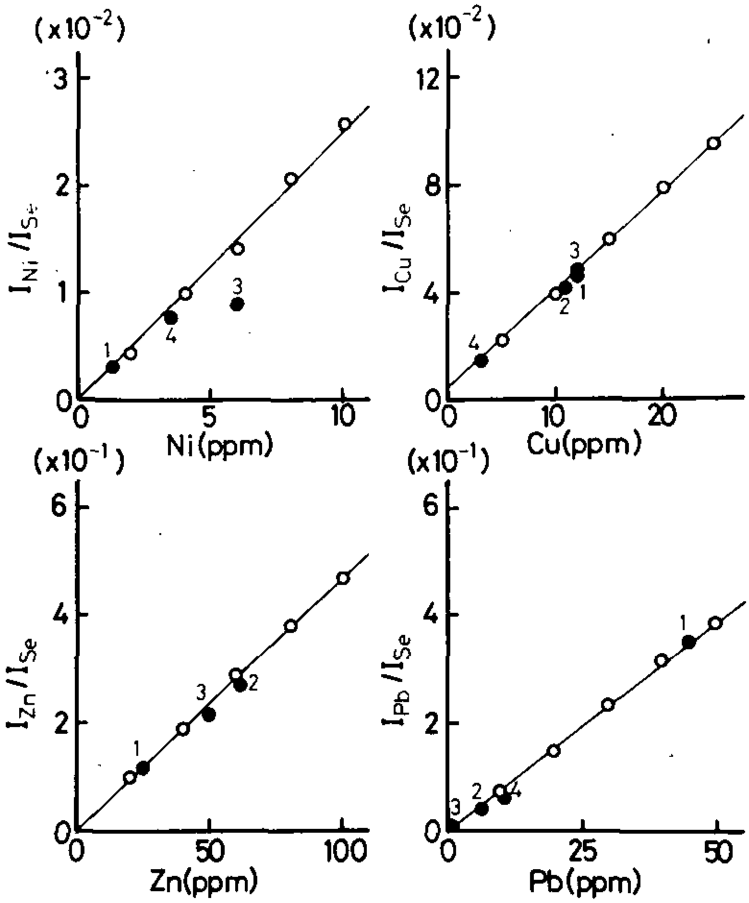


Fig. 3. Calibration curves
Open symbol : Artificial reference standard
Closed symbol : NBS standard reference material
(1: Orchard Leaves, 2: Tomato Leaves, 3: Spinach, 4: Pine Needles)

### *Results*

The contents of K, Ca, Mn, Fe, Ni, Cu, Zn, As, Pb, Rb and Sr in NIES Pepperbush are shown in Table II. The analytical results are presented as the average values of 5 runs.

**Table II.**
Analytical Values for NIES SRM Pepperbush by Energy-Dispersive X-ray Fluorescence Spectrometry

| Element | Content (%) | Element | Content (μg/g) |
|---|---|---|---|
| K | 1.32 | Mn | 2180 |
| Ca | 1.36 | Fe | 284 |
| | | Ni | 8.2 |
| | | Cu | 11.1 |
| | | Zn | 362 |
| | | As | 3.8 |
| | | Pb | 5.6 |
| | | Rb | 74.0 |
| | | Sr | 34.2 |

# CHAPTER VII

# DETERMINATION OF ALKALIES, ALKALINE EARTHS AND SOME HEAVY METALS IN PEPPERBUSH BY ISOTOPE DILUTION MASS SPECTROMETRY

M. Murozumi, S. Nakamura, T. Igarashi,
K. Suga and K. Yoshida

### *Abstract*

Isotope dilution surface ionization mass spectrometry has been applied to the accurate determination of K, Rb, Mg, Ca, Sr, Ba, Cu, Cd, Pb, Tl and Ag in Pepperbush. After spiking with a stable isotope of one of the above components under measurement, the spiked sample is decomposed in a mixture of $HNO_3$, $HClO_4$ and HF at low temperature under pressure. The decomposed sample is heated to dryness and the residue obtained is dissolved in dilute $HNO_3$ solution. An aliquot of this solution is applied to the mass spectrometer (Hitachi-RMU6) equipped with a surface ionization device incorporating a Re single filament. The analyses for alkalies or alkaline earth elements are performed by loading directly an aliquot of the digest onto the filament, while the heavy metals are determined after the component has been extracted from the digest as the dithizonate in $CHCl_3$.

By using a composite spike solution, several components, for example Cu, Cd, Tl and Pb, are determined simultaneously.

The present method can measure minute amounts, i.e., ng amounts, of the above elements with an accuracy of 1%.

### *Introduction*

### 1-1 Isotope Dilution Method

When a weighed amount of Pepperbush has reached isotopic equilibration with a known amount of a spike, the following relation exits between the molar amounts of the element and its isotopes:

$${}^{i}M/{}^{i'}M = \frac{m_n \times {}^{i}f_n + m_s \times {}^{i}f_s}{m_n \times {}^{i'}f_n + m_s \times {}^{i'}f_s} \quad \text{......................} (1)$$

where ${}^{i}M$ and ${}^{i'}M$ represent the molar amounts of isotope i and isotope i' comprising the element under measurement, $m_n$ and $m_s$ are the molar amounts of the element in the sample and of the added spike, and ${}^{i}f_n$ and ${}^{i}f_s$ are the isotopic abundances of the isotope i of the element in the sample and the spike respectively. The amount $m_n$ can be determined by measuring the $^{i}M/^{i'}M$ ratio when $^{i}f_n$, $^{i}f_s$, $^{i'}f_n$, $^{i'}f_s$ and $m_s$ are known. As an example, the following equation (2) holds for the lead isotopes in the sample equilibrated with the $^{206}Pb$ spike,

$$^{208}Pb/^{206}Pb = \frac{m_{Pb\text{-sample}} \times {}^{208}f_{Pb\text{-sample}} + m_{Pb\text{-spike}} \times {}^{208}f_{Pb\text{-spike}}}{m_{Pb\text{-sample}} \times {}^{206}f_{Pb\text{-sample}} + m_{Pb\text{-spike}} \times {}^{206}f_{Pb\text{-spike}}} \quad \text{............} \quad (2)$$

The numbers for $^{208}f_{Pb\text{-sample}}$, $^{208}f_{Pb\text{-spike}}$, $^{206}f_{Pb\text{-sample}}$, $^{206}f_{Pb\text{-spike}}$ and $m_{Pb\text{-spike}}$ are listed in Table I. One of the following lead spikes, $^{204}Pb$, $^{207}Pb$, $^{208}Pb$ and $^{205}Pb$ can be used for the $^{206}Pb$ spike, the choice depending on the lead isotopic abundance of the sample.

### 1.2 Spikes

The spikes used were imported from the Oak Ridge National Laboratory, Tenn., U.S.A. and each spike was dissolved in dilute $HNO_3$ and stored in a tightly stoppered teflon or quartz bottle. Molar concentrations of these spike solutions, $m_s$, were standardized to reference materials and the concentrations are compiled in Table II.

Composite spike solutions were prepared by mixing some of the spike solutions as shown in Table III.

### 1.3 Mass Spectrometer

A Hitachi RMU6 type mass spectrometer manufactured in 1968 is equipped with an ion source for multipurpose measurements. A simplified block diagram of the instrument is given in Fig. 1. A Re single filament is used as the ion emitter for measurement of the components in Pepperbush.

The instrument can detect an emitter current of $10^{-19}$A for the mass spectrum emitted by an element, and measure the isotopic ratio with an accuracy of 0.n% in the coefficient of variation.

### 1.4 Laboratory

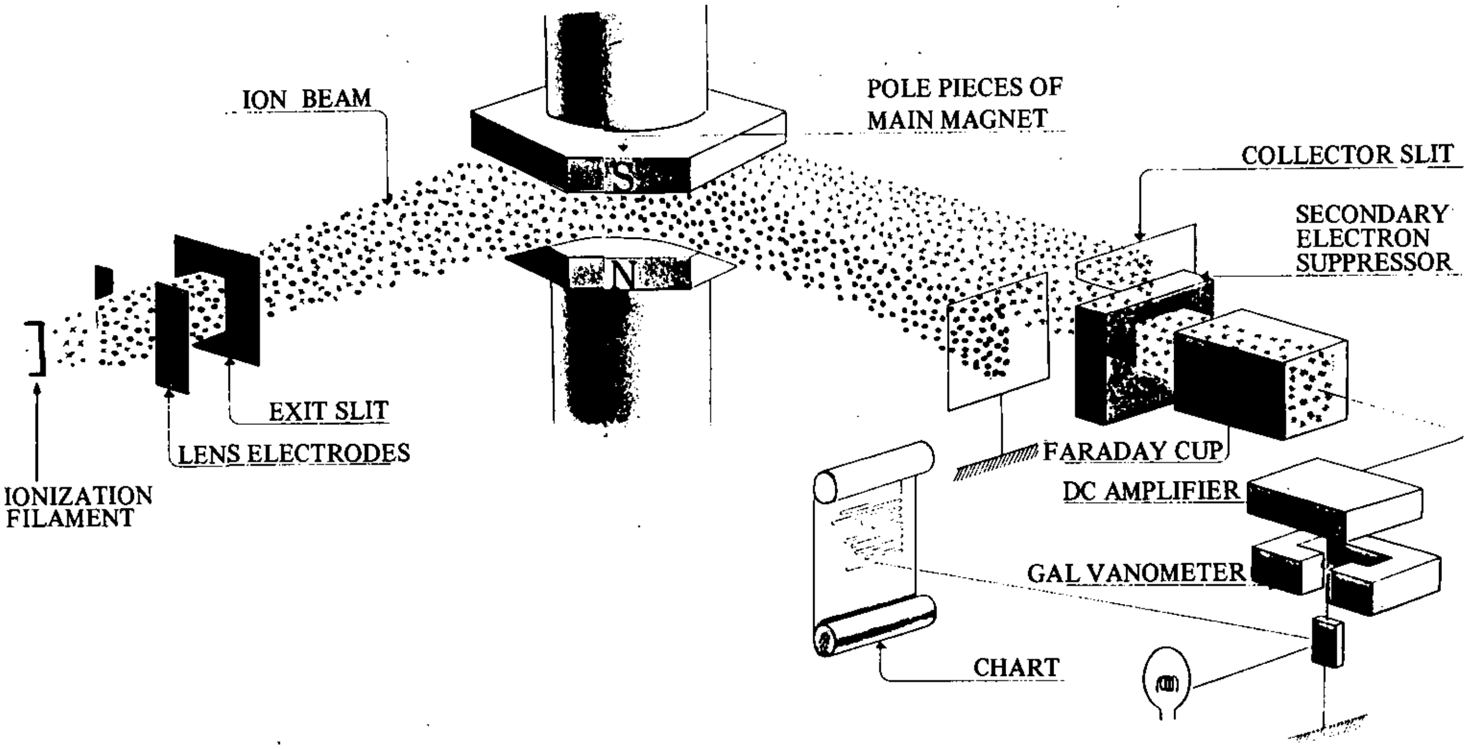
Reagents and samples were all prepared in a class 100 clean draft chamber and on class 100 clean benches set in a class 300 clean room, the latter being constructed of stainless steel and having a methoxy-resin covered floor. Contamination from the room environment and reagents is, for example in the case of lead analysis, at the level of 1.0 ng for one determination of this element in Pepperbush, corresponding to only one thousandth of the measured value, and is 2.0 ng for copper, 0.3 ng for cadmium and 0.06 ng for thallium respectively.

## *Experiments*

### 2.1 Reagents

#### 2.1.1 Purification of common reagents and water

Reagents were all purified by sub-boiling distillation (twice) of analytical grade



Ion trajectory: Radius 200 *mm*, deflection angle 90°
250 *mm* and 45° for electrostatic sector attached

Fig. 1 Schematic diagram of mass spectrometer (Hitachi RMU6).

**Table I.** Isotopic Abundances of Some Natural Elements and Spikes.

| | Natural | $^{203}$Tl Spike |
|---|---|---|
| $^{203}$Tl | 0.2966 ± 0.0004 | 0.9497 ± 0.0006 |
| $^{205}$Tl | 0.7034 ± 0.0004 | 0.0503 ± 0.0006 |
| At.Wt.* | 204.4 | 203.1 |

| | Natural | $^{65}$Cu Spike |
|---|---|---|
| $^{63}$Cu | 0.692 ± 0.0001 | 0.0030 ± 0.00002 |
| $^{65}$Cu | 0.308 ± 0.0001 | 0.9970 ± 0.0001 |
| At.Wt.* | 63.5 | 64.9 |

| | Natural | Pepperbush | $^{206}$Pb Spike |
|---|---|---|---|
| $^{204}$Pb | 0.0139 | 0.0137 | - |
| $^{206}$Pb | 0.2509 | 0.2511 | 0.9998 |
| $^{207}$Pb | 0.2138 | 0.2141 | 0.0000834 |
| $^{208}$Pb | 0.5215 | 0.5211 | 0.000127 |
| At.Wt.* | 207.2 | | 206.2 |

| | Natural | $^{116}$Cd Spike |
|---|---|---|
| $^{106}$Cd | 0.0127 | - |
| $^{108}$Cd | 0.0090 | - |
| $^{110}$Cd | 0.125 | 0.00213 |
| $^{111}$Cd | 0.129 | 0.00239 |
| $^{112}$Cd | 0.241 | 0.00514 |
| $^{113}$Cd | 0.123 | 0.00339 |
| $^{114}$Cd | 0.287 | 0.0132 |
| $^{116}$Cd | 0.0748 | 0.974 |
| At.Wt.* | 112.3 | 115.8 |

| | Natural | $^{87}$Rb Spike |
|---|---|---|
| $^{85}$Rb | 0.722 | 0.0266 |
| $^{87}$Rb | 0.279 | 0.973 |
| At.Wt.* | 85.6 | 86.8 |

| | Natural | $^{86}$Sr Spike |
|---|---|---|
| $^{84}$Sr | 0.00560 | - |
| $^{86}$Sr | 0.0936 | 0.976 |
| $^{87}$Sr | 0.0702 | 0.00674 |
| $^{88}$Sr | 0.826 | 0.0172 |
| At.Wt.* | 87.6 | 85.9 |

| | Natural | $^{135}$Ba Spike |
|---|---|---|
| $^{130}$Ba | 0.0011 | 0.0001 |
| $^{132}$Ba | 0.0010 | 0.0001 |
| $^{134}$Ba | 0.0243 | 0.0046 |
| $^{135}$Ba | 0.0658 | 0.935 |
| $^{136}$Ba | 0.0783 | 0.0163 |
| $^{137}$Ba | 0.112 | 0.0089 |
| $^{138}$Ba | 0.717 | 0.0355 |
| At.Wt.* | 137.5 | 135.1 |

| | Natural | $^{41}$K Spike |
|---|---|---|
| $^{39}$K | 0.931 | 0.0080 |
| $^{40}$K | 0.000118 | - |
| $^{41}$K | 0.0688 | 0.992 |
| At.Wt.* | 39.1 | 40.9 |

| | Natural | $^{42}$Ca Spike |
|---|---|---|
| $^{40}$Ca | 0.970 | 0.0483 |
| $^{42}$Ca | 0.00640 | 0.945 |
| $^{43}$Ca | 0.00145 | 0.00093 |
| $^{44}$Ca | 0.0206 | 0.00577 |
| $^{46}$Ca | 0.000033 | - |
| $^{48}$Ca | 0.00135 | - |
| At.Wt.* | 41.9 | 40.1 |

| | Natural | $^{26}$Mg Spike |
|---|---|---|
| $^{24}$Mg | 0.792 | 0.00284 |
| $^{25}$Mg | 0.0997 | 0.00109 |
| $^{26}$Mg | 0.108 | 0.996 |
| At.Wt.* | 24.3 | 26.0 |

* atomic weight.

**Table II.**
Concentrations of Spike Stock Solutions.

| Spike | Total Concentration ($\mu$g/g) | Concentration ($\mu$mol/g) Total | Each Spike | |
|---|---|---|---|---|
| $^{206}$Pb | 382.0 | 1.844 | $^{206}$Pb | 1.843 |
| | | | $^{208}$Pb | 0.0002 |
| $^{65}$Cu | 119 | 1.834 | $^{63}$Cu | 0.00550 |
| | | | $^{65}$Cu | 1.828 |
| $^{203}$Tl | 197 | 0.970 | $^{203}$Tl | 0.921 |
| | | | $^{205}$Tl | 0.0488 |
| $^{116}$Cd | 238.0 | 2.055 | $^{114}$Cd | 0.02713 |
| | | | $^{116}$Cd | 2.002 |
| $^{41}$K | 359.6 | 8.792 | $^{39}$K | 0.07034 |
| | | | $^{41}$K | 8.722 |
| $^{87}$Rb | 188.0 | 2.166 | $^{85}$Rb | 0.05761 |
| | | | $^{87}$Rb | 2.107 |
| $^{26}$Mg | 420.4 | 16.17 | $^{24}$Mg | 0.04592 |
| | | | $^{26}$Mg | 16.10 |
| $^{42}$Ca | 251.4 | 6.269 | $^{40}$Ca | 0.03028 |
| | | | $^{42}$Ca | 5.924 |
| $^{86}$Sr | 250 | 2.910 | $^{86}$Sr | 2.841 |
| | | | $^{88}$Sr | 0.050 |
| $^{135}$Ba | 202.9 | 1.502 | $^{135}$Ba | 1.404 |
| | | | $^{138}$Ba | 0.053 |
| $^{107}$Ag | 389.3 | 3.642 | $^{107}$Ag | 3.577 |
| | | | $^{109}$Ag | 0.06446 |

## Table III.
Concentrations of Composite Spike Solutions.

| | | |
|---|---|---|
| **A Composite $^{65}$Cu, $^{116}$Cd, $^{206}$Pb and $^{203}$Tl Spike Solution.** | | |
| $^{65}$Cu spike | Total Cu = 7.584 (μg/g) = 0.1169 (μmol/g) | 0.1165 (μmol/g) as $^{65}$Cu |
| $^{116}$Cd spike | Total Cd = 1.145 (μg/g) = 0.009888 (μmol/g) | 0.009631 (μmol/g) as $^{116}$Cd |
| $^{206}$Pb spike | Total Pb = 7.396 (μg/g) = 0.03590 (μmol/g) | 0.03590 (μmol/g) as $^{206}$Pb |
| $^{203}$Tl spike | Total Tl = 1.250 (μg/g) = 0.006154 (μmol/g) | 0.005845 (μmol/g) as $^{203}$Tl |
| **A Composite $^{87}$Rb, $^{86}$Sr and $^{138}$Ba Spike Solution.** | | |
| $^{87}$Rb spike | Total Rb = 3.030 (μg/g) = 0.03491 (μmol/g) | 0.03397 (μmol/g) as $^{87}$Rb |
| $^{86}$Sr spike | Total Sr = 3.284 (μg/g) = 0.03823 (μmol/g) | 0.03731 (μmol/g) as $^{86}$Sr |
| $^{135}$Ba spike | Total Ba = 5.982 (μg/g) = 0.04423 (μmol/g) | 0.03171 (μmol/g) as $^{135}$Ba |
| **A Composite $^{41}$K, $^{42}$Ca and $^{26}$Mg Spike Solution.** | | |
| $^{41}$K spike | Total K = 245.3 (μg/g) = 5.998 (μmol/g) | 5.950 (μmol/g) as $^{41}$K |
| $^{42}$Ca spike | Total Ca = 251.4 (μg/g) = 6.269 (μmol/g) | 5.924 (μmol/g) as $^{42}$Ca |
| $^{26}$Mg spike | Total Mg = 98.60 (μg/g) = 3.792 (μmol/g) | 3.777 (μmol/g) as $^{26}$Mg |

reagents using teflon or quartz stills. The purified reagents were stored in tightly stoppered teflon bottles, and water was stored in quartz bottles. The concentrations of impurities in the purified reagents were determined by the application of isotope dilution surface ionization mass spectrometry, the results being compiled in Table IV.

**Table IV.**
Heavy Metal Impurities in Purified Reagents and Water, ng/kg.

| | Tl | Cu | Cd | Pb |
|---|---|---|---|---|
| 14M $HNO_3$ | 0.384±0.031 | 23.6±0.6 | 0.537±0.030 | 3.83±0.07 |
| 6M HCl | 0.872±0.072 | 3.78±0.25 | 0.306±0.076 | 1.68±0.04 |
| 20% $NH_4OH$ | 0.111±0.011 | 86.6 ±0.9 | 0.68 ±0.15 | 3.56±0.23 |
| 46% HF | 1.90 ±0.13 | 47.4 ±0.4 | 0.411±0.081 | 4.08±0.07 |
| 60% $HClO_4$ | 0.111±0.011 | 151 ±1 | 1.40 ±0.09 | 20.1 ±0.2 |
| $H_2O$ | 0.118±0.012 | 3.10±0.10 | 0.191±0.035 | 1.61±0.04 |

### 2.1.2 Spikes

Previously weighed spikes, $^{41}$K, $^{87}$Rb, $^{26}$Mg, $^{42}$Ca, $^{86}$Sr, $^{135}$Ba, $^{65}$Cu, $^{116}$Cd, $^{206}$Pb, $^{203}$Tl and $^{107}$Ag were individually dissolved in 5% $HNO_3$ and stored in tightly stoppered teflon bottles. The concentration of every spike solution was standardized relative to reference materials of known isotopic abundance and concentration. All working spike solutions were prepared by diluting the stock solution with dilute $HNO_3$. The isotopic abundance, $^{i}f_{spike}$, and the molar concentration, $m_{spike}$, of the spike solutions are compiled in Tables I and II. Sometimes various composite spike solutions were prepared by mixing two or more spike solutions. These were conveniently used for the simultaneous determination of elements in Pepperbush. The data of $^{i}f_{spike}$ and $m_{spike}$ for some composite spikes are listed in Table III. The use of a mixed spike solution is applicable only when the composite spike does not suffer from mutual chemical contamination. For example, a mixed spike solution of $^{65}$Cu, $^{116}$Cd, $^{203}$Tl and $^{206}$Pb suffers a minute Cu contamination resulting from the $^{116}$Cd, $^{203}$Tl and $^{206}$Pb spikes as shown in Table V; the degree of contamination, however, does not prevent the accurate determination of the copper content of Pepperbush.

A composite spike of $^{87}$Rb, $^{86}$Sn and $^{135}$Ba suffers no such contamination as illustrated by the data in Table VI.

### 2.1.3 Cleaning

Bottles, beakers and other apparati were dipped into a warm concentrated $HNO_3$ solution for several days. After being rinsed with purified water several times, the apparati were kept dipped in purified water before use.

The ionization device of the mass spectrometer was dismantled and cleaned with $HNO_3$ prior to use. The newly assembled device was electrically ignited for three hours in a vacuum chamber to expel contaminants.

**Table V.**

Contamination in 1.000 g of a Composite Spike Solution of $^{65}$Cu, $^{116}$Cd, $^{206}$Pb and $^{203}$Tl.

|  |  | μmol/g-spike solution |  |
|---|---|---|---|
| $^{65}$Cu | Theoretical | $^{65}$Cu | 0.1165 |
|  | Contaminatant (measured) | $^{65}$Cu | 0.0000045 |
|  |  | $^{63}$Cu | 0.000010 |
| $^{116}$Cd | Theoretical | $^{116}$Cd | 0.009631 |
|  | Contaminatant (measured) | $^{116}$Cd | 0.00000008 |
|  |  | $^{114}$Cd | 0.00000031 |
| $^{206}$Pb | Theoretical | $^{206}$Pb | 0.03590 |
|  | Contaminatant (measured) | $^{206}$Pb | 0.0000003 |
|  |  | $^{208}$Pb | 0.0000007 |
| $^{203}$Tl | Theoretical | $^{203}$Tl | 0.005845 |
|  | Contaminatant (measured) | $^{203}$Tl | 0.00000012 |
|  |  | $^{205}$Tl | 0.00000028 |

**Table VI.**

Contamination in 1.000 g of a Composite Spike Solution of $^{87}$Rb, $^{86}$Sr and $^{135}$Ba.

|  |  | μmol/g-spike solution |  |
|---|---|---|---|
| $^{87}$Rb | Theoretical | $^{87}$Rb | 0.03397 |
|  |  | $^{85}$Rb | 0.0009285 |
|  | After Composited (measured) | $^{87}$Rb | 0.03398 |
|  |  | $^{85}$Rb | 0.0009268 |
| $^{86}$Sr | Theoretical | $^{86}$Sr | 0.03731 |
|  |  | $^{88}$Sr | 0.0000658 |
|  | After Composited (measured) | $^{86}$Sr | 0.03732 |
|  |  | $^{88}$Sr | 0.0000657 |
| $^{135}$Ba | Theoretical | $^{135}$Ba | 0.04140 |
|  |  | $^{138}$Ba | 0.0001572 |
|  | After Composited (measured) | $^{135}$Ba | 0.04139 |
|  |  | $^{138}$Ba | 0.000158 |

## 2.2 Isotope Dilution Mass Spectrometry

### 2.2.1 Simultaneous Determination of Copper[1,2,3], Cadmium[4], Thallium[5] and Lead[6,7,8] in Pepperbush

(1) Sample Preparation -1

To three aliquots of Pepperbush (Run Nos. 1, 2, and 3 in Table VII), were added nearly equal amounts of a quadri-spiked solution containing $^{65}$Cd, $^{116}$Cd, $^{203}$Tl and $^{206}$Pb. Each spiked sample was decomposed in a mixture of $HNO_3$ (3.0 ml), $HClO_4$ (1.0 ml) and HF (1.0 ml)) in a teflon vessel, under pressure. The temperature for decomposition was carefully maintained below 160 ℃. The digest in the vessel changed to a transparent solution in two hours. Each solution thus obtained was heated to dryness at atmospheric pressure and an additional 0.3 ml of $HNO_3$ was added to the residue and the mixture was evaporated to dryness again to ensure that the decomposition reaction was complete. The white residue was dissolved in 6.0 ml of 2M $HNO_3$, and transferred to a teflon separating funnel. The pH of the solution was adjusted to pH2 by the addition of $NH_4OH$. Copper in the solution was extracted into 10 ml of 0.0013% dithizone-$CHCl_3$. The dithizone-$CHCl_3$ layer containing copper dithizonate was transferred to a second teflon separating funnel.

For extraction of Cd, Tl and Pb, the acidic solution in the first separating funnel was neutralized with the addition of $NH_4OH$ to a pH value of 8.5. After 1.0 ml of 50% $(NH_4)_2$-Citrate and 0.50 ml of 2% KCN were added as the masking reagents for foreign ions, cadmium, thallium and lead were extracted and trasnsferred to the separating funnel containing the copper dithizonate-$CHCl_3$ solution. The subsequent solution was mixed well by swirling. The mixed dithizone-$CHCl_3$ solution was rinsed twice with 5.0 ml portions of purified water to remove the aqueous emulsion containing the major components of Pepperbush and the masking reagents. 5.0 ml of 7M $HNO_3$ was added to the dithizone-$CHCl_3$ solution for back extraction of the elements. After the dithizone-$CHCl_3$ solution was discarded, the acid layer was rinsed twice with 5.0 ml portions of $CHCl_3$ solution. The acid solution was transferred to a teflon beaker and 1.0 ml of $HNO_3$ and 0.25 ml of $HClO_4$ were added. The contents were gently heated under a pure $N_2$ atmosphere on an electric heater to dryness. Subsequently, 0.3 ml of $HNO_3$ and 0.1 ml of $HClO_4$ were added to the residue and the contents were heated again to completely decompose the organic material.

The residue thus obtained was dissolved in a mixed solution of 65$\mu$l of 0.02% silica gel suspension aqueous solution and 5$\mu$l of 2.0% $H_3PO_4$ solution which functioned as the stabilizing reagents for ion beam emissions of the four elements on the ionization device in the mass spectrometer.

(2) Sample Preparation -2

Sample preparation 2 was identical to the above except that three samples were prepared having different $^{i}M/^{i'}M$ ratios. That is, the only difference between Sample Preparation 1 and 2 was the amount of added spikes as shown for Run Nos. 4, 5 and 6 in Table VII. From the analytical point of view, Sample Preparation 2 provided a better $^{i}M/^{i'}M$ ratio for the four elements for solving of equation (1). The analytical results are given in Table VII.

## 2.3 Determination of Silver[9]

### 2.3.1 Isotopic Abundance of Natural and Spike Silver

The isotopic ratio value of natural silver (99.99% grade commercial silver metal), $^{109}$Ag/$^{107}$Ag, could be determined with an accuracy of 0.1 - 0.2% (coefficient of variation),

## Table VII

Simultaneous Determination of Thallium, Copper, Lead and Cadmium in Pepperbush using a Quadri-spike Solution

| | Run No. | Sample taken (g) | Spike added $^{203}\mathrm{Tl}$ (µmol) | $^{205}\mathrm{Tl}$ (µmol) | Filament current (A) | $\frac{^{205}\mathrm{Tl}}{^{203}\mathrm{Tl}}$ | C.V.* (%) | Element found $^{203}\mathrm{Tl}$ (nmol) | $^{205}\mathrm{Tl}$ (nmol) | Tl (µg) | Tl concn. (ppm) | Average concentration (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tl | 1 | 0.1259 | 0.01186 | 0.00063 | 0.880 | 0.0576 | 0.1 | 0.0238 | 0.0564 | 0.0164 | 0.130 | 0.139±0.013 |
| | 2 | 0.1615 | 0.01136 | 0.00060 | 0.770 | 0.0594 | 0.2 | 0.0316 | 0.0754 | 0.0218 | 0.135 | |
| | 3 | 0.2122 | 0.01178 | 0.00062 | 0.774 | 0.0622 | 0.9 | 0.0469 | 0.111 | 0.0323 | 0.152 | |
| | 4 | 0.1429 | 0.00605 | 0.00032 | 0.790 | 0.0633 | 0.1 | 0.0270 | 0.0640 | 0.0186 | 0.125 | 0.129±0.004 |
| | 5 | 0.2056 | 0.00609 | 0.00032 | 0.790 | 0.0677 | 0.4 | 0.0389 | 0.0921 | 0.0268 | 0.130 | |
| | 6 | 0.2497 | 0.00606 | 0.00032 | 0.780 | 0.0711 | 0.1 | 0.0477 | 0.113 | 0.0329 | 0.132 | |

| | | | $^{65}\mathrm{Cu}$ (µmol) | $^{63}\mathrm{Cu}$ (µmol) | | $\frac{^{63}\mathrm{Cu}}{^{65}\mathrm{Cu}}$ | | $^{65}\mathrm{Cu}$ (µmol) | $^{63}\mathrm{Cu}$ (µmol) | Cu (µg) | Tl concn. (ppm) | Average Concn. (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cu | 1 | | 0.2352 | 0.0007 | 1.090 | 0.0675 | 1.9 | 0.00699 | 0.0157 | 1.44 | 11.4 | 11.4±0.1 |
| | 2 | | 0.2253 | 0.0007 | 1.040 | 0.0833 | 0.5 | 0.00893 | 0.0201 | 1.84 | 11.4 | |
| | 3 | | 0.2338 | 0.0007 | 1.120 | 0.1090 | 1.0 | 0.0116 | 0.0262 | 2.40 | 11.3 | |
| | 4 | | 0.1201 | 0.0004 | 1.180 | 0.1486 | 0.7 | 0.00835 | 0.0188 | 1.72 | 11.5 | 11.4±0.2 |
| | 5 | | 0.1208 | 0.0004 | 1.130 | 0.1947 | 0.3 | 0.0111 | 0.0250 | 2.29 | 11.2 | |
| | 6 | | 0.1203 | 0.0004 | 1.140 | 0.2371 | 0.5 | 0.0146 | 0.0311 | 2.90 | 11.6 | |

| | Run No. | Sample taken (g) | Spike added $^{206}Pb$ (μmol) | Spike added $^{208}Pb$ (μmol) | Filament current (A) | $\frac{^{208}Pb}{^{206}Pb}$ | C.V.* (%) | Element found $^{208}Pb$ (μmol) | Element found $^{206}Pb$ (μmol) | Element found Pb (μg) | Pb concn. (ppm) | Average Concn. (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 0.07285 | 0.00001 | 1.340 | 0.02300 | 2.7 | 0.00323 | 0.000691 | 0.670 | 5.32 | 5.54±0.23 |
| | 2 | | 0.06974 | 0.00001 | 1.350 | 0.03317 | 0.7 | 0.00449 | 0.000960 | 0.931 | 5.77 | |
| Pb | 3 | | 0.07236 | 0.00001 | 1.480 | 0.04031 | 0.5 | 0.00565 | 0.00121 | 1.17 | 5.53 | |
| | 4 | | 0.03717 | 0.000004 | 1.500 | 0.05251 | 0.3 | 0.00383 | 0.000819 | 0.794 | 5.32 | 5.35±0.04 |
| | 5 | | 0.03741 | 0.000004 | 1.520 | 0.07133 | 0.5 | 0.00531 | 0.00114 | 1.10 | 5.33 | |
| | 6 | | 0.03724 | 0.000004 | 1.430 | 0.08714 | 0.4 | 0.00652 | 0.00139 | 1.35 | 5.39 | |

| | | | $^{116}Cd$ (μmol) | $^{114}Cd$ (μmol) | | $\frac{^{114}Cd}{^{116}Cd}$ | | $^{116}Cd$ (μmol) | $^{114}Cd$ (μmol) | Cd (μg) | Cd concn. (ppm) | Average concn. (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 0.01934 | 0.00026 | 1.450 | 0.1161 | 1.6 | 0.000538 | 0.00207 | 0.808 | 6.42 | 6.58±0.17 |
| | 2 | | 0.01871 | 0.00025 | 1.480 | 0.1544 | 1.8 | 0.000705 | 0.00271 | 1.06 | 6.58 | |
| Cd | 3 | | 0.01941 | 0.00026 | 1.640 | 0.1928 | 1.1 | 0.000951 | 0.00364 | 1.43 | 6.75 | |
| | 4 | | 0.00998 | 0.000135 | 1.500 | 0.2511 | 0.2 | 0.000659 | 0.00253 | 0.991 | 6.64 | 6.66±0.03 |
| | 5 | | 0.01003 | 0.000136 | 1.520 | 0.3337 | 0.2 | 0.000918 | 0.00353 | 1.38 | 6.69 | |
| | 6 | | 0.00999 | 0.000135 | 1.610 | 0.3942 | 0.4 | 0.00110 | 0.00425 | 1.66 | 6.64 | |

* coefficient of variation.

while those of $^{107}$Ag and $^{109}$Ag spikes were 0.2 - 0.5% as shown in Table VIII. The isotopic abundances of the substances are compiled in Table VIII.1.

The intensity of the $^{107}Ag^+$ ion beam emitted from the silver compound on the ionization device listed in Table VIII.3, means that the detection limit of the present method is $10^{-12}$ g for this element.

### 2.3.2 Sample Preparation

Pepperbush samples of different amounts were placed in two teflon beakers. A certain amount of the $^{107}$Ag spike (Table VIII.2), 3.0 ml of $HNO_3$ and 1.0 ml of HF were added to each sample. Each sample was gently heated for 3 hours in a teflon bomb supplied with a purified $N_2$ stream. After the complete decomposition of the sample, the transparent solution which remained in the beaker was evaporated to dryness at atmospheric pressure. The residue was dissolved in 10 ml of 1 M $HNO_3$ and the solution was transferred to a teflon separating funnel. Silver was extracted from the acid solution into 10 ml of 0.0013% dithizone-$CHCl_3$, and then the organic solution containing silver dithizonate was washed twice with 10 ml portions of purified water. Then, silver was back-extracted into 6.0 ml of 7M $HNO_3$. The nitric acid solution was evaporated to dryness. The extraction procedure was repeated again to eliminate completely the presence of foreign ions.

Finally, the dried residue was dissolved in a mixed stabilizing solution of silica gel and $H_3PO_4$ and an aliquot of the solution was applied to the mass spectrometer for $Ag^+$ emission measurement. The results are presented in Table VIII.

## 2.4. Simultaneous Determination of Rubidium, Strontium and Barium$^{10)}$

### 2.4.1 IsotopicAbundances of the Natural Elements and Spikes

The isotopic abundances of natural rubidium, strontium and barium, and of their spikes are listed in Table I. The concentration of a composite spike solution is given in Table III.

### 2.4.2 Sample Preparation

Two samples of different amounts were added to two teflon beakers. A certain amount of a composite triple spike solution containing $^{87}$Rb, $^{86}$Sr, and $^{135}$Ba was added to each beaker. The contents were decomposed in a mixed acid solution of 3.0 ml of $HNO_3$, 1.0 ml of 60% $HClO_4$ and 0.5 ml of 46% HF, under pressure. The transparent solution thus obtained was heated to dryness at atmospheric pressure. The residue was dissolved in 0.1 ml of purified water. An aliquot of this aqueous solution (ca. 0.01 ml) was loaded onto the rhenium ionization filament for mass spectrometry. The use of silica gel and $H_3PO_4$ stabilizers was unnecessary for the emission of these ions on the rhenium filament, unlike the case of the above-mentioned heavy metals.

The mass spectrum of $Rb^+$ appears first at a lower temperature, from the ionization device, then with increasing temperature $Sr^+$ followed by $Ba^+$ are detected. This means that the isotopic ratios of $^{85}Rb^+/^{87}Rb^+$, $^{88}Sr^+/^{86}Sr^+$ and $^{138}Ba^+/^{135}Ba^+$ can be determined successively at different temperatures of the ionization filament, and potential mutual interference is avoided. That is to say, for the measurement of the $^{85}Rb^+/^{87}Rb^+$ ratio, the overlapping $^{87}Sr^+$ spectrum is not observed, because the higher filament temperature is necessary for strontium emission.

The mass spectrum of $Ba^+$ appears at the highest filament temperature after the complete decay of $Rb^+$ and $Sr^+$. The analytical results are given in Table IX.

## Table VIII.

Determination of Silver by Isotope Dilution Mass Spectrometry.

1) Isotope Abundance of Natural Silver, $^{107}$Ag and $^{109}$Ag spikes.

| | Muroran Institute of Technology | | Other Institutes | |
|---|---|---|---|---|
| | $^{107}$Ag | $^{109}$Ag | $^{107}$Ag | $^{109}$Ag |
| Natural Ag | 52.07±0.02 | 47.93±0.02* | 51.95±0.08 | 48.05±0.09** |
| $^{107}$Ag spike | 98.23±0.01 | 1.77±0.01 | 98.22±0.05 | 1.78±0.05*** |
| $^{109}$Ag spike | 0.73±0.00 | 99.27±0.00 | 0.74±0.05 | 99.26±0.05*** |

* Four-9 Grade Commercial Ag metal.
** U.S. National Bureau of Standards.
*** Oak Ridge National Laboratory.

2) Concentration of $^{107}$Ag and $^{109}$Ag Spike Solutions.

| | Total Concentration (μg/g) | $^{107}$Ag (μg/g) | $^{107}$Ag (μmol/g) | $^{109}$Ag (μg/g) | $^{109}$Ag (μmol/g) |
|---|---|---|---|---|---|
| $^{107}$Ag spike | 0.3538±0.0014 | 0.3476 | $3.251 \times 10^{-3}$ | 0.0063 | $5.858 \times 10^{-5}$ |
| $^{109}$Ag spike | 0.1850±0.0013 | 0.0013 | $1.2 \times 10^{-5}$ | 0.1837 | $1.686 \times 10^{-3}$ |

3) Determination of Silver in Orchard Leaves (N.B.S) and Pepperbush.

| Sample | Sample taken (g) | $^{107}$Ag spike added (μg) | $^{107}$Ag$^{+}$ ion intensity (A) | $^{109}$Ag/$^{107}$Ag | C.V.* (%) | Total Ag found (ng) | Ag concentration (ng/g) |
|---|---|---|---|---|---|---|---|
| Orchard Leaves | 0.1866 | 0.5461 | $1.73 \times 10^{-12}$ | 0.02257 | 0.3 | 5.269±0.70 | 28.24±0.04 |
| Pepperbush (I) | 0.3238 | 0.3647 | $3.1 \times 10^{-15}$ | 0.03212 | 0.2 | 11.02±0.06 | 34.04±0.19 |
| Pepperbush (II) | 0.6061 | 0.3596 | $3.7 \times 10^{-13}$ | 0.04484 | 0.1 | 20.97±0.03 | 34.60±0.05 |

* coefficient of variation.

**Table IX**

Simultaneous Determination of Rubidium, Strontium and Barium in Pepperbush using a Triple Spike Solution.

| Run No. | Sample taken (g) | $^{87}$Rb spike added ($\mu$g) | Filament current (A) | $\frac{^{85}\text{Rb}}{^{87}\text{Rb}}$ | C.V.* (%) | Rubidium found ($\mu$g) | Rubidium concentration ($\mu$g/g) | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.1086 | 9.008 | 0.670 | 0.5574 | 0.2 | 8.08±0.02 | 74.4±0.2 | 74.1±0.4 |
| 2 | 0.2178 | 8.842 | 0.555 | 0.9122 | 0.2 | 16.0 ±0.1 | 73.7±0.2 | |

| Run No. | Sample taken (g) | $^{86}$Sr spike added ($\mu$g) | Filament current (A) | $\frac{^{88}\text{Sr}}{^{86}\text{Sr}}$ | C.V.* (%) | Strontium found ($\mu$g) | Strontium concentration ($\mu$g/g) | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.1086 | 9.763 | 2.330 | 0.3433 | 0.3 | 4.00±0.01 | 36.8±0.1 | 36.6±0.2 |
| 2 | 0.2178 | 9.582 | 2.360 | 0.6513 | 0.6 | 7.94±0.05 | 36.4±0.3 | |

| Run No. | sample taken (g) | $^{135}$Ba spike added ($\mu$g) | Filament current (A) | $\frac{^{138}\text{Ba}}{^{135}\text{Ba}}$ | C.V.* (%) | Barium found ($\mu$g) | Barium concentration ($\mu$g/g) | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.1086 | 17.78 | 2.020 | 0.7601 | 0.6 | 18.2 ±0.2 | 168±1 | 166±3 |
| 2 | 0.2178 | 17.46 | 2.560 | 1.382 | 0.5 | 35.5 ±0.2 | 163±1 | |

* coefficient of variation

## Table X

Successive Determination of Potassium, Magnesium and Calcium in Pepperbush using a Triple Spike Solution.

| Run No. | Sample taken (g) | Aliquot of dissolved sample, spiked (g) | $^{41}$K spike added (μg) | Filament current (A) | $\frac{^{39}K}{^{41}K}$ | C.V.* (%) | Potassium found (μg) | Potassium concentration (%) | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.105 | 0.01109 | 730.7 | 0.650 | 0.2171 | 0.9 | 159±1 | 1.43±0.01 | 1.42±0.01 |
| 2 | 1.105 | 0.02270 | 735.9 | 0.480 | 0.4211 | 0.3 | 320±1 | 1.41±0.01 | |

| Run No. | Sample taken (g) | Aliquot of dissolved sample, spiked (g) | $^{26}$Mg spike added (μg) | Filament current (A) | $\frac{^{24}Mg}{^{26}Mg}$ | C.V.* (%) | Magnesium found (μg) | Magnesium concentration (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 1.105 | 0.02456 | 481.7 | 2.040 | 0.1506 | 1.9 | 85.8±1.8 | 0.348±0.008 |

| Run No. | Sample taken (g) | Aliquot of dissolved sample, spiked (g) | $^{42}$Ca spike added (μg) | Filament current (A) | $\frac{^{40}Ca}{^{42}Ca}$ | C.V.* (%) | Calcium found (μg) | Calcium concentration (%) | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.105 | 0.01109 | 772.0 | 2.230 | 0.2602 | 0.4 | 150±1 | 1.35±0.01 | 1.36±0.01 |
| 2 | 1.105 | 0.02270 | 813.9 | 1.780 | 0.4578 | 0.5 | 308±2 | 1.36±0.01 | |

* coefficient of variation

## 2.5 Simultaneous Determination of Potassium11), Calcium12,13) and Magnesium

A triple spike solution of $^{41}K$, $^{26}Mg$ and $^{42}Ca$ was added to the sample. The spiked sample was prepared by the same procedure as for the Rb, Sr and Ba determinations. The mass spectra of $K^+$, $Ca^+$ and $Mg^+$ appeared successively with increasing temperature of the ionization filament and the respective isotopic ratios were determined successively thus avoiding the potential mutual interference. The analytical results are given in Table X.

### *Conclusion*

Isotope dilution surface ionization mass spectrometry is continuing to attract the attentions of scientists due to its inherent accuracy. The analytical results for the present method and other common methods are compiled in Table XI. Although failing in analytical capability for volatile components, for example, P, S, Se, Te and As essential for environmental research, the present method can determine much more elements than those listed in Table XI, when certain prerequisites are satisfied. The method requires a specific machine, clean rooms and ultra-pure reagents and special techniques are necessary for optimum performance. With the best efforts of workers, however, the present method will reveal its superior ability to give the most accurate results in environmental analyses.

**Table XI**

Data by Isotope Dilution Mass Spectrometry and Other Common Methods.
Concentrations of Some Components in Pepperbush.

| | Cu (ppm) | Cd (ppm) | Tl (ppm) | Pb (ppm) |
|---|---|---|---|---|
| IDMS | 11.4±0.7 | 6.66±0.03 | 0.129±0.004 | 5.35±0.04 |
| Others | 7.2~12.5 | 6.4~6.9 | - | 6.22 |
| Average* | 11.4 | 6.7 | - | - |

| | Ag (ppb) | K (%) | Ca (%) | Rb (ppm) |
|---|---|---|---|---|
| IDMS | 34.3±0.3 | 1.42±0.01 | 1.36±0.01 | 74.1±0.4 |
| Others | - | 1.41~1.83 | 1.22~1.53 | 72~80.1 |
| Average* | - | 1.54 | 1.43 | 76 |

| | Sr (ppm) | Ba (ppm) | Mg (ppm) | |
|---|---|---|---|---|
| IDMS | 36.6±0.2 | 166± 3 | 3480± 80 | |
| Others | 35.6~37.1 | 154~180 | 3590~4540 | |
| Average* | 36.9 | 170 | 3940 | |

* average of others.

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# CHAPTER VIII

# THE CERTIFICATION OF PEPPERBUSH*

K. Okamoto

## *Introduction*

In the previous chapters, the nature of Pepperbush plant, the preparation and analyses by four analytical techniques were described. In this chapter, we shall outline the procedures taken to enable the certification of Pepperbush. The initial stage of the certification process requires analytical data for the various elements to be obtained from independent and established analytical techniques. A collaborative study on the elemental analysis of Pepperbush has been carried out with a number of Japanese scientists and the analytical data obtained by various analytical techniques are first presented. The certification of elemental composition has been performed using analytical data obtained at NIES and by the collaborating laboratories. The criteria for certification of elemental composition, and the certification process for Pepperbush are described. The certified and reference values for Pepperbush are also listed.

## *1. Analytical Techniques Employed for Elemental Analysis of Pepperbush*

Analyses of Pepperbush have been performed using various analytical techniques at 28 laboratories. The analytical techniques used for the elements are summarized in Table I. At NIES, atomic absorption spectrometry (AAS), flame emission spectrometry (FES), inductively coupled plasma emission spectrometry (ICP) and X-ray fluorescence spectrometry (XRF) have been employed. Analytical reslults by the above techniques and by isotope dilution mass spectrometry (IDMS), instrumental neutron activation analysis (INAA), neutron activation analysis with radiochemical separation (RNAA), instrumental photon activation analysis (IPAA), substoichiometric isotope dilution analysis (Sub IDA), spectrophotometry (SP), spectrofluorimetry (SF), gravimetry (Grav) and potentiometry (Pot) have also been provided by collaborating laboratories. More than 40 elements in Pepperbush have been determined by 12 independent analytical techniques.

## *2. Cooperating Laboratories*

Table II indicates the principal investigators, addresses and code numbers for the

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* Presented in part at Joint US-Japan Symposium on Standard Reference Materials, Koriyama, 1979.

collaborating laboratories, including members of staff of NIES.

**Table I**

Analytical Techniques Used for Elements

| Analytical Technique | Element |
|---|---|
| Atomic absorption spectrometry (AAS) | Al, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Rb, Sr, Zn |
| Flame emission spectrometry (FES) | Ca, K, Na, Rb, Sr |
| Inductively coupled plasma emission spectrometry (ICP) | Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Sc, Sr, Ti, V, Y, Zn |
| X-ray fluorescence spectrometry (XRF) | As, Ca, Cd, Cu, Fe, K, Mn, Ni, Pb, Rb, Sr, Zn |
| Isotope dilution mass spectrometry (IDMS) | Ag, Ba, Ca, Cd, Cu, K, Mg, Pb, Rb, Sr, Tl |
| Neutron activation analysis | |
| Instrumental (INAA) | Al, As, Ba, Br, Ca, Cd, Cl, Co, Cr, Cs, Cu, Eu, Fe, Ge, Hf, K, La, Mg, Mn, Na, Rb, Sb, Sc, Se, Sm, Ta, Th, V, W, Zn |
| Radiochemical separation (RNAA) | As, Cl, I, Sb |
| Instrumental photon activation analysis (IPAA) | As, Ba, Ca, Mg, Mn, Na, Ni, Rb, Sr, Zn, Zr |
| Substoichiometric isotope dilution analysis (Sub IDA) | Fe |
| Spectrophotometry (SP) | Co, Cr, Fe, Ni, P |
| Spectrofluorimetry (SF) | Se |
| Gravimetry (Grav) | Ca, Si |
| Potentiometry (Pot) | Cl |

### *3. Analytical Values for Pepperbush*

Table III compiles the analytical values for Pepperbush, the analytical techniques employed and the code number of the collaborating participants.

The analytical procedures employed at NIES and those for IDMS have been reported in detail in Chapter IV - VII. The cooperating laboratories were requested to perform determinations for elements which they considered they were equipped to determine. The results received from the participating laboratories were normalized whenever necessary, for example, to the appropriate units. In compiling the data the following procedures were adopted:

(1) All values given in Table III were taken from the original reports of the participants with

## Table II

Names and Affiliations of Collaborating Analysts

| Laboratory Code | Principal Investigator | Affiliation |
|---|---|---|
| 01 | K. Okamoto | National Institute for Environmental Studies, Chemistry & Physics Division, Ibaraki 305. |
| 02 | C.W. McLeod | *ibid.* |
| 03 | N. Furuta | *ibid.* |
| 04 | T. Takamatsu | *ibid.* , Water & Soil Division, Ibaraki 305. |
| 05 | H. Akaiwa | Gunma Univ., Dept. of Chemistry, Kiryu, Gunma 376. |
| 06 | M. Ambe | Sagami Chemical Research Centre, Nishi-Ohnuma, 4-4-1, Sagamishi, Kanagawa 229. |
| 07 | M. Ichikuni | Tokyo Inst. of Technology, Dept. of Envir. Chem. & Eng., Nagatsuka, Midoriku, Yokohama 227. |
| 08 | S. Iwata | Kyoto Univ., Research Reactor Inst., Div. of Hot Lab., Kumatoricho, Sennangun, Osaka 590-04. |
| 09 | M. Kamada | Kagoshima Univ., Chemical Inst., Koorimoto, 1-21-35, Kagoshima 890. |
| 10 | K. Kimura | Aoyama Gakuin Univ., College of Sci. & Tech., 16-1, Chitosedai 6, Setagaya, Tokyo 157. |
| 11 | N. Suzuki | Tohoku Univ., Dept. of Chemistry, Aoba, Sendai 980. |
| 12 | T. Sotobayashi | Niigata Univ., Dept. of Chemistry, Igarashi, Niigata 950-21. |
| 13 | Y. Takashima | Kyushu Univ., Dept. of Chemistry, Hakozaki, Higashiku, Fukuoka 812. |
| 14 | K. Terada | Kanazawa Univ., Dept. of Chemistry, Marunouchi, 1-1, Kanazawa 920. |
| 15 | K. Nagashima | Univ. of Tsukuba, Dept. of Chemistry, Sakuramura, Ibaraki 305. |
| 16 | S. Nagatsuka | Tokyo Metropolitan Isotope Research Centre, 2-11-1, Fukazawa, Setagayaku, Tokyo 158. |
| 17 | Y. Nishikawa | Kinki Univ., Dept. of Chemistry, Kowakae, Higashi-Osaka, Osaka 577. |
| 18 | Y. Hashimoto | Keio Univ., Dept. of Applied Chemistry, Hiyoshi, Kohokuku, Yokohama 223. |
| 19 | H. Hamaguchi | Japan Chemical Analysis Centre, 295-3, Sannocho, Chibashi, Chiba 281. |
| 20 | M. Furukawa | Nagoya Univ., Dept. of Chemistry, Nagoya, Aichi 464. |
| 21 | Y. Murakami | Tokyo Metropolitan Univ., Dept. of Chemistry, Fukazawa 1, Setagaya, Tokyo 158. |
| 22 | M. Murozumi | Muroran Inst. of Technology, Dept. of Applied Chemistry, Mizumotocho, 271, Muroranshi 050. |
| 23 | N. Yamagata | Inst. of Public Health, Dept. of Radiological Health, 6-1, Shiroganedai, 4, Minatoku, Tokyo 108. |
| 24 | K. Watanuki | Tokyo Univ., College of General Education, Dept. of Chem., Komaba, 3-8-1, Meguroku, 153. |
| 25 | K. Kudo | Ibaraki Electrical Comm. Lab., Nippon Telegraph & Telephone Public Corp., Tokai, Ibaraki. |
| 26 | A. Ward | Fisher Scientific Co., Jarrell-Ash Div., 590 Lincoln St., Waltham, Mass. 02154, U.S.A. |
| 27 | N. Kokubu | University of Electro-Communications, 1-5-1, Chofugaoka, Chofushi, Tokyo 182. |
| 28 | J.S. Jacobson | Boyce Thompson Inst. for Plant Research, Cornell Univ., Ithaca, New York 14853, U.S.A. |

as little change as possible; significant figures of the values given in the Table are identical to those of the data reported by the participants.

(2) Data which was reported based on the 'silica-gel' dry weight (mean moisture content, 7.5%), were normalized to that based on drying in an air-oven at 85 ℃ for 4 hrs (mean moisture content, 8.0%).

(3) When a laboratory employed two or more independent analytical techniques for the same elements, the mean value produced by each technique was treated and presented separately.

(4) Disregarding differences in detailed procedures existing among individual laboratories which used the same method, the averages of the values obtained by each techniques were calculated.

### *4. Certified Values for Pepperbush*

"In the development of each SRM, assurance must be obtained that the material used is uniform and stable, that test methods yield repeatable and consistent results, and that the conditions under which the material is to be used are carefully described. Eventually, these qualitative statements will have to be translated into quantitative terms, using data generated from the tests, and condensed into a certificate that will be understandable and useful to the user."1) Therefore, the data obtained by various analytical techniques are processed and condensed into the form presented on the certificate, where the numerical values are expressed into two parts: the certificate value of the property and the uncertainty of this value.

Here we need to consider the reliability of analytical results which is a function of accuracy and precision (reproducibility). The precision of results in terms of the standard deviation can readily be determined for analytical methods by internal measurements. The determination of accuracy, however, is no easy task and the evaluation of each analytical method with respect to their inherent accuracy is indispensable if certification is to be achieved. On the basis of pure statistical considerations it is difficult to judge which value is closest to the 'true value'. At the National Bureau of Standards (USA), analytical methods are classified considering their accuracy into the following categories: (1) definitive method, (2) reference method, (3) field method. "A definitive method is one in which all major significant parameters have been related by direct or a solid chain of evidence to the base or derived units of the SI"2) (International System of Units). At the National Bureau of Standards, isotope dilution mass spectrometry has been used extensively as a definitive method for the accurate measurement of inorganic constituents at trace levels. As already shown in the equation (1) (see page 60) in the previous chapter, the inherent advantages of the IDMS are: "First, all chemical manipulations are done on a weight basis and involve straight-forward stoichiometric separations, precipitations, etc, to determine $m_{spike}$ and $m_{sample}$. Second, the mass spectrometric determinations involve only ratios and not the absolute determinations of the isotopes involved. Therefore, no instrumental correction or

## Table III

Analytical Values ($\mu$g/g) for Pepperbush

| Element | Value† ($\mu$g/g) | Technique | Laboratory Code |
|---|---|---|---|
| Ag | 0.034 | IDMS | 22 |
| Al | 431 | ICP | 02 |
| | 447 | ICP | 03 |
| | 345 | AAS | 07 |
| | 436 | INAA | 08 |
| | 700 | INAA | 10 |
| | 356 | ICP | 15 |
| | 600 | INAA | 16 |
| | 582 | INAA | 18 |
| | 747 | INAA | 19 |
| | 549 | INAA | 20 |
| | 648 | INAA | 21 |
| | 509 | AAS | 23 |
| | 321 | ICP | 26 |
| As | 3.8 | XRF | 04 |
| | 2.0 | AAS | 06 |
| | 2.2 | IPAA | 11 |
| | 2.3 | RNAA | 14 |
| | 1.7 | INAA | 16 |
| | 2.23 | INAA | 18 |
| | 2.8 | INAA | 19 |
| | 2.20 | INAA | 20 |
| | 3.15 | INAA | 21 |
| | 2.4 | INAA | 23 |
| | 2.41 | INAA | 25 |
| | 1.5 | ICP | 26 |
| B | 48 | ICP | 26 |
| Ba | 164 | ICP | 02 |
| | 173 | ICP | 03 |
| | 160 | IPAA | 11 |
| | 158 | ICP | 15 |
| | 160 | INAA | 16 |
| | 180 | INAA | 18 |
| | 180 | INAA | 19 |
| | 186 | INAA | 20 |
| | 113 | INAA | 21 |
| | 166 | IDMS | 22 |
| | 164 | ICP | 26 |
| Br | 2.0 | INAA | 08 |
| | 1.4 | INAA | 16 |
| | 3.4 | INAA | 19 |
| | 1.60 | INAA | 20 |
| | 2.06 | INAA | 21 |
| | 6.36 | INAA | 25 |

| Element | Value† (μg/g) | Technique | Laboratory Code |
|---|---|---|---|
| C | 48.1 (Wt. %) | * | 06 |
| | 47.8 | * | 13 |
| | 45.8 | * | 17 |
| | 47.3 | * | 18 |
| | 46.2 | * | 18 |
| Ca | 1.32 (Wt. %) | AAS | 01 |
| | 1.35 | FES | 01 |
| | 1.44 | ICP | 02 |
| | 1.49 | ICP | 03 |
| | 1.36 | XRF | 04 |
| | 1.38 | AAS | 07 |
| | 1.24 | INAA | 08 |
| | 1.45 | IPAA | 11 |
| | 1.45 | AAS | 12 |
| | 1.37 | AAS | 14 |
| | 1.40 | ICP | 15 |
| | 1.4 | INAA | 16 |
| | 1.5 | INAA | 18 |
| | 1.37 | Grav | 19 |
| | 1.39 | AAS | 19 |
| | 1.32 | INAA | 20 |
| | 1.55 | INAA | 21 |
| | 1.36 | IDMS | 22 |
| | 1.51 | AAS | 24 |
| | 1.35 | ICP | 26 |
| Cd | 6.7 | AAS | 01 |
| | 7.3 | ICP | 02 |
| | 6.7 | AAS | 05 |
| | 6.5 | AAS | 07 |
| | 6.8 | AAS | 09 |
| | 6.9 | AAS | 10 |
| | 6.8 | AAS | 12 |
| | 6.9 | AAS | 13 |
| | 6.9 | AAS | 14 |
| | 7.3 | INAA | 19 |
| | 7.6 | AAS | 19 |
| | 6.64 | IDMS | 22 |
| | 6.87 | XRF | 22 |
| | 6.2 | ICP | 26 |
| Cl | 203 | Pot | 05 |
| | 207 | INAA | 08 |
| | 195 | Pot | 14 |
| | 220 | INAA | 16 |
| | 188 | RNAA | 19 |
| Co | 23.7 | AAS | 01 |
| | 21.4 | ICP | 02 |
| | 19.8 | ICP | 03 |
| | 27 | AAS | 05 |
| | 20.9 | INAA | 10 |

| Element | Value† (μg/g) | Technique | Laboratory Code |
|---|---|---|---|
| Co | 22 | AAS | 12 |
| | 18.9 | SP | 13 |
| | 21.5 | AAS | 14 |
| | 21.0 | ICP | 15 |
| | 21 | INAA | 16 |
| | 24.2 | INAA | 18 |
| | 22 | INAA | 19 |
| | 23.8 | AAS | 19 |
| | 21.9 | INAA | 20 |
| | 20.1 | INAA | 21 |
| | 24 | AAS | 22 |
| | 26.0 | INAA | 23 |
| | 27 | AAS | 24 |
| | 23.6 | INAA | 25 |
| | 20.4 | ICP | 26 |
| Cr | 1.1 | AAS | 12 |
| | 2.3 | SP | 13 |
| | 1.1 | INAA | 16 |
| | 1.38 | SP | 17 |
| | 3.8 | INAA | 19 |
| | 1.18 | INAA | 20 |
| | 1.30 | INAA | 21 |
| | 1.1 | INAA | 23 |
| | 1.31 | INAA | 25 |
| | 2.0 | ICP | 26 |
| Cs | 1.1 | INAA | 16 |
| | 1.31 | INAA | 18 |
| | 1.3 | INAA | 19 |
| | 1.12 | INAA | 20 |
| | 1.22 | INAA | 21 |
| Cu | 12.1 | AAS | 01 |
| | 11.3 | ICP | 02 |
| | 11.6 | ICP | 03 |
| | 11.1 | XRF | 04 |
| | 11.8 | AAS | 05 |
| | 12.1 | AAS | 07 |
| | 12.4 | AAS | 09 |
| | 13.0 | AAS | 10 |
| | 12.6 | AAS | 12 |
| | 13.2 | AAS | 14 |
| | 12.2 | ICP | 15 |
| | 13.0 | AAS | 19 |
| | 13.4 | INAA | 21 |
| | 11.4 | IDMS | 22 |
| | 11.6 | XRF | 22 |
| | 11.9 | ICP | 26 |
| Eu | 0.038 | INAA | 19 |
| | 0.0083 | INAA | 25 |

| Element | Value† (μg/g) | Technique | Laboratory Code |
|---|---|---|---|
| F | 3.6 | Pot | 27 |
|  | 6, 12 | SP | 28 |
| Fe | 205 | AAS | 01 |
|  | 204 | ICP | 02 |
|  | 217 | ICP | 03 |
|  | 284 | XRF | 04 |
|  | 190 | AAS | 05 |
|  | 180 | AAS | 07 |
|  | 144 | INAA | 08 |
|  | 226 | AAS | 10 |
|  | 210 | INAA | 10 |
|  | 194.0 | Sub IDA | 11 |
|  | 208 | AAS | 12 |
|  | 252 | SP | 13 |
|  | 202 | AAS | 14 |
|  | 203 | ICP | 15 |
|  | 210 | INAA | 16 |
|  | 260 | SP | 17 |
|  | 220 | INAA | 18 |
|  | 200 | INAA | 19 |
|  | 211 | AAS | 19 |
|  | 207 | INAA | 20 |
|  | 260 | INAA | 21 |
|  | 226 | AAS | 22 |
|  | 207 | SP | 24 |
|  | 193 | INAA | 25 |
|  | 196 | ICP | 26 |
| Ge | 1.2 | INAA | 10 |
| H | 5.67 (Wt. %) | * | 06 |
|  | 5.52 | * | 13 |
|  | 5.88 | * | 17 |
|  | 5.56 | * | 18 |
|  | 5.46 | * | 18 |
| Hf | 0.23 | INAA | 19 |
| Hg | 0.056 | AAS | 06 |
|  | 0.059 | AAS | 06 |
|  | 0.057 | AAS | 09 |
|  | 0.045 | AAS | 13 |
|  | 0.053 | AAS | 23 |
| I | 0.66 | RNAA | 19 |
| K | 1.53 (Wt. %) | AAS | 01 |
|  | 1.53 | FES | 01 |
|  | 1.49 | ICP | 02 |
|  | 1.32 | XRF | 04 |
|  | 1.52 | AAS | 07 |
|  | 1.61 | INAA | 08 |
|  | 1.57 | AAS | 10 |
|  | 1.60 | INAA | 10 |

| Element | Value† ($\mu$g/g) | Technique | Laboratory Code |
|---|---|---|---|
| K | 1.54 (Wt. %) | AAS | 12 |
| | 1.53 | AAS | 13 |
| | 1.53 | FES | 14 |
| | 1.45 | ICP | 15 |
| | 1.5 | INAA | 16 |
| | 1.77 | INAA | 18 |
| | 1.46 | INAA | 19 |
| | 1.51 | FES | 19 |
| | 1.57 | INAA | 20 |
| | 1.52 | INAA | 21 |
| | 1.42 | IDMS | 22 |
| | 1.60 | AAS | 24 |
| | 1.51 | INAA | 25 |
| | 1.42 | ICP | 26 |
| La | 0.29 | INAA | 16 |
| | 0.40 | INAA | 18 |
| | 0.29 | INAA | 19 |
| | 0.28 | INAA | 20 |
| Mg | 0.416 (Wt. %) | AAS | 01 |
| | 0.426 | ICP | 02 |
| | 0.412 | ICP | 03 |
| | 0.387 | AAS | 07 |
| | 0.38 | AAS | 10 |
| | 0.35 | INAA | 10 |
| | 0.372 | IPAA | 11 |
| | 0.390 | AAS | 12 |
| | 0.384 | AAS | 14 |
| | 0.368 | ICP | 15 |
| | 0.42 | INAA | 16 |
| | 0.354 | AAS | 17 |
| | 0.42 | INAA | 18 |
| | 0.366 | INAA | 19 |
| | 0.411 | AAS | 19 |
| | 0.347 | IDMS | 22 |
| | 0.427 | AAS | 24 |
| | 0.404 | ICP | 26 |
| Mn | 0.210 (Wt. %) | AAS | 01 |
| | 0.209 | ICP | 02 |
| | 0.211 | ICP | 03 |
| | 0.218 | XRF | 04 |
| | 0.194 | AAS | 05 |
| | 0.202 | AAS | 07 |
| | 0.218 | INAA | 08 |
| | 0.179 | INAA | 10 |
| | 0.204 | IPAA | 11 |
| | 0.194 | AAS | 12 |
| | 0.201 | AAS | 14 |
| | 0.185 | ICP | 15 |
| | 0.170 | INAA | 16 |
| | 0.200 | AAS | 17 |

| Element | Value† (μg/g) | Technique | Laboratory Code |
|---|---|---|---|
| Mn | 0.200 | INAA | 18 |
| | 0.204 | INAA | 19 |
| | 0.205 | AAS | 19 |
| | 0.214 | INAA | 20 |
| | 0.197 | INAA | 21 |
| | 0.182 | AAS | 22 |
| | 0.196 | AAS | 24 |
| | 0.174 | INAA | 25 |
| | 0.201 | ICP | 26 |
| Mo | 0.4 | ICP | 26 |
| N | 1.36 (Wt. %) | * | 06 |
| | 1.50 | * | 13 |
| | 1.45 | * | 17 |
| | 1.53 | * | 18 |
| | 1.58 | * | 18 |
| Na | 101 | AAS | 01 |
| | 106 | FES | 01 |
| | 107 | ICP | 02 |
| | 98 | AAS | 07 |
| | 140 | INAA | 08 |
| | 119 | INAA | 10 |
| | 115 | IPAA | 11 |
| | 87 | FES | 14 |
| | 120 | INAA | 16 |
| | 120 | INAA | 18 |
| | 112 | INAA | 19 |
| | 100 | INAA | 20 |
| | 160 | INAA | 21 |
| | 118 | AAS | 22 |
| | 86 | AAS | 24 |
| | 108 | AAS | 25 |
| | 95 | ICP | 26 |
| Ni | 8.7 | AAS | 01 |
| | 8.4 | ICP | 02 |
| | 8.2 | XRF | 04 |
| | 8.8 | AAS | 05 |
| | 8.1 | AAS | 10 |
| | 8.7 | IPAA | 11 |
| | 11.6 | SP | 13 |
| | 8.1 | AAS | 14 |
| | 8.9 | ICP | 15 |
| | 9.95 | AAS | 19 |
| | 9 | AAS | 22 |
| P | 0.103 (Wt. %) | ICP | 02 |
| | 0.097 | ICP | 03 |
| | 0.113 | SP | 06 |
| | 0.111 | SP | 07 |
| | 0.109 | SP | 10 |
| | 0.106 | ICP | 15 |

| Element | Value† ($\mu$g/g) | Technique | Laboratory Code |
|---|---|---|---|
| P | 0.106 (Wt.%) | SP | 24 |
| | 0.107 | ICP | 26 |
| Pb | 5.6 | AAS | 01 |
| | 5.6 | XRF | 04 |
| | 7.2 | AAS | 05 |
| | 6.26 | AAS | 09 |
| | 6.9 | AAS | 14 |
| | 6.3 | AAS | 19 |
| | 5.33 | IDMS | 22 |
| | 5.4 | XRF | 22 |
| | 4.9 | ICP | 26 |
| Rb | 75.0 | AAS | 01 |
| | 74.7 | FES | 01 |
| | 74.0 | XRF | 04 |
| | 75 | INAA | 08 |
| | 73.0 | IPAA | 11 |
| | 74 | INAA | 16 |
| | 110 | FES | 19 |
| | 82.9 | INAA | 20 |
| | 73 | INAA | 21 |
| | 73.9 | IDMS | 22 |
| Sb | 0.16 | RNAA | 14 |
| | 0.13 | INAA | 16 |
| | 0.147 | AAS | 18 |
| | 0.19 | INAA | 19 |
| | 0.16 | INAA | 20 |
| | 0.10 | INAA | 21 |
| Sc | 0.027 | INAA | 08 |
| | 0.050 | INAA | 16 |
| | 0.066 | INAA | 18 |
| | 0.048 | INAA | 19 |
| | 0.048 | INAA | 20 |
| | 0.049 | INAA | 21 |
| | 0.065 | INAA | 25 |
| | 0.05 | ICP | 26 |
| Se | 0.123 | SF | 17 |
| | 0.148 | SF | 17 |
| | 0.15 | INAA | 19 |
| | 0.41 | INAA | 21 |
| | 0.2 | ICP | 26 |
| $SiO_2$ | 0.53 (Wt.%) | Grav | 07 |
| | 0.37 | Grav | 10 |
| | 0.41 | Grav | 15 |
| | 0.36 | SP | 17 |
| | 0.42 | Grav | 19 |
| | 0.39 | Grav | 24 |
| Sm | 0.049 | INAA | 16 |
| | 0.050 | INAA | 18 |

| Element | Value† (μg/g) | Technique | Laboratory Code |
|---|---|---|---|
| Sm | 0.059 | INAA | 19 |
| | 0.043 | INAA | 20 |
| Sr | 30.0 | FES | 01 |
| | 34.2 | ICP | 02 |
| | 34 | ICP | 03 |
| | 34.2 | XRF | 04 |
| | 39.8 | AAS | 07 |
| | 36.3 | IPAA | 11 |
| | 35.8 | AAS | 13 |
| | 39.0 | ICP | 15 |
| | 40 | AAS | 19 |
| | 36.5 | IDMS | 22 |
| | 35 | ICP | 26 |
| Ta | 0.13 | INAA | 19 |
| Th | 0.11 | INAA | 19 |
| Ti | 4.3 | ICP | 26 |
| Tl | 0.129 | IDMS | 22 |
| V | 0.58 | INAA | 10 |
| | 0.23 | ICP | 26 |
| W | 0.28 | INAA | 18 |
| Y | 0.64 | ICP | 26 |
| Zn | 340 | AAS | 01 |
| | 339 | ICP | 02 |
| | 333 | ICP | 03 |
| | 362 | XRF | 04 |
| | 330 | AAS | 05 |
| | 337 | AAS | 07 |
| | 290 | INAA | 08 |
| | 336 | AAS | 09 |
| | 360 | AAS | 10 |
| | 330 | IPAA | 11 |
| | 360 | AAS | 12 |
| | 290 | AAS | 14 |
| | 347 | ICP | 15 |
| | 330 | INAA | 16 |
| | 380 | INAA | 18 |
| | 400 | INAA | 18 |
| | 330 | INAA | 19 |
| | 353 | AAS | 19 |
| | 362 | INAA | 20 |
| | 393 | INAA | 21 |
| | 360 | AAS | 22 |
| | 410 | INAA | 23 |
| | 363 | INAA | 25 |
| | 328 | ICP | 26 |
| Zr | 0.8 | IPAA | 11 |
| | 0.5 | ICP | 26 |

† based on dry weight (air-oven 85 ℃ for 4 hrs).

* CHN analyser.

errors are involved".2) However, definitive methods are expensive, time-consuming, and often require special apparatus and highly skilled scientists so that the widespread use of definitive method has been precluded. These are the reasons why a Chapter on IDMS (Chapter VII) is included in this report.

"A reference method is a method of proven accuracy. Here the accuracy of the method rests on or is demonstrated usually by (but not always) a definitive method. Reference methods are generally arrived at by consensus. That is, fairly extensive testing of the accuracy claims are made by a number of laboratories before its status is accepted by the measurement laboratories that will be using the method."2) The accuracy of a reference method can also be assayed via a SRM(s) whose property(ies) have been accurately determined by a definitive method.

The classification of analytical methods by the National Bureau of Standards is reasonable and practically useful for the certification process. The criteria used for certification of elemental composition of Pepperbush, which correspond to those used at the National Bureau of Standards, are as follows: (1) the first criterion is that analytical values should be determined "by a definitive method", for instance, IDMS and gravimetry. Two or more independent analyses by the definitive methods are, however, required. Or (2) the second criterion is "by two or more independent reference methods", for example, AAS and INAA.

We basically adopted these criteria for the certification of the elemental composition of Pepperbush. However, the IDMS results provided have not completely satisfied the first item because the data represents replicates at one laboratory only. Also the elements determined by IDMS were limited owing to the principle and capability of the method. In order to certify the elemental composition of Pepperbush, therefore, we adopted the values determined not only by IDMS but also by reference methods. For those elements where values were not determined by the definitive method, the analytical values determined by two or more reference methods were adopted for certification.

Table IV shows the certified elements and the analytical techniques used for the determination of the elements in Peppperbush. The definitive method, that is IDMS, has been employed to determine K, Ca, Mg, Ba, Rb, Sr, Cu, Cd and Pb. Three or more independent reference methods have also been used for each element certified. Atomic absorption spectrometry has been used most frequently. Neutron activation analysis has been frequently used for multielement analysis. It is noteworthy that inductively coupled plasma emission spectrometry has been employed for simultaneous multielement analysis.

Next, we need to illustrate how certification was performed. Let's consider copper. There were 16 independent analytical values for copper determined by AAS, ICP, IDMS, INAA and XRF, as presented in Table III. The entire range of the copper content lies between 11.1 and 13.4 ppm. The overall mean and standard deviation are computed to be 12.2 and 0.7 ppm, respectively, therefore, the overall mean ±2 times the standard deviation gives 12.2±1.4 ppm.

Here we need to explain how we estimated the uncertainties or the overall errors of the certified values. Uncertainty includes sample variability, measurement errors and possible bias among analytical methods. As mentioned in Chapter III, the homogeneity test for certain elements indicated that sample variability among bottles may be considered negligible. Therefore, we should consider measurement errors and systematic errors among methods.

Grubbs's statistical outlier test3) was first applied to determine whether any results should be rejected. Fortunately, there was no outlier for copper, therefore, all values listed here were used for further statistical treatment.

Table IV

Analytical Techniques Used for Elemental Analysis of Pepperbush

| | |
|---|---|
| MAJOR CONSTITUENTS | |
| Potassium | AAS, FES, ICP, XRF, IDMS, INAA |
| Calcium | AAS, FES, ICP, XRF, IDMS, INAA, IPAA, Grav. |
| MINOR CONSTITUENTS | |
| Magnesium | AAS, ICP, IDMS, INAA, IPAA |
| Manganese | AAS, ICP, XRF, INAA, IPAA. |
| TRACE CONSTITUENTS | |
| Zinc | AAS, ICP, XRF, INAA, IPAA |
| Iron | AAS, ICP, XRF, INAA, Sub IDA, SP |
| Barium | ICP, IDMS, INAA, IPAA |
| Sodium | AAS, FES, ICP, INAA, IPAA |
| Rubidium | AAS, FES, XRF, IDMS, INAA, IPAA |
| Strontium | AAS, FES, ICP, XRF, IDMS, IPAA |
| Cobalt | AAS, ICP, INAA, SP |
| Copper | AAS, ICP, XRF, IDMS, INAA |
| Nickel | AAS, ICP, XRF, IPAA, SP |
| Cadmium | AAS, ICP, XRF, IDMS, INAA |
| Lead | AAS, ICP, XRF, IDMS |
| Arsenic | ICP, XRF, INAA, RNAA, IPAA |

Abbreviations used for analytical techniques are indicated in Table I.

Using the example of copper, the estimation of the accuracy and precision will be explained. Fig. 1 shows the entire range (1), the overall mean ±2 times the standard deviation (2), the 95% confidence interval computed from the AAS results (3) which includes the variation within and between laboratories. The 95% confidence intervals computed from IDMS (4) and ICP (5) results obtained at one laboratory are also indicated in Fig. 1.

Second, we should consider so-called possible bias or systematic error among analytical methods. In Fig. 1, the centre of the horizontal lines indicates the mean for each method. It is apparent that a small bias exists between the three methods (3) (4) (5). Just now, however, there is not enough data to estimate bias among the methods. One way is to use the mean obtained by the definitive method as a true value and then, to estimate the accuracy of the other methods. As for the copper content of Pepperbush, more independent results by IDMS could make this possible. In this case, based on consideration of the overall mean ±2 times the standard deviation and the 95% confidence intervals for the three methods, the copper content of Pepperbush has been certified to be 12±1 ppm.

The certified values for Pepperbush are given in Table V and the criteria for certification were similar to that used in the case of Cu, namely the certified values are based on consideration of the overall mean ±2 times the standard deviation or the 95% confidence limits for the various methods.

### 5. *Reference Values for Pepperbush*

The reference values for Pepperbush are also included in Table V. The reference values are defined as those values which were consistent but were obtained by only one reference method, or in the case of two reference methods used, if some analytical problems were suspected. For example, Cr and Cs were determined by only INAA, and Hg was determined by only AAS. Tl was determined by only IDMS. All these cases need more independent analyses. (Table III already compiles the up-to-date analytical results so that these situation for the elements will change in the future). As for P, the results by SP and ICP were close, however, further examination of the sample dissolution procedure is required since loss of P is suspected. We hope that with additional data reference values could resort to certified values at a later date.

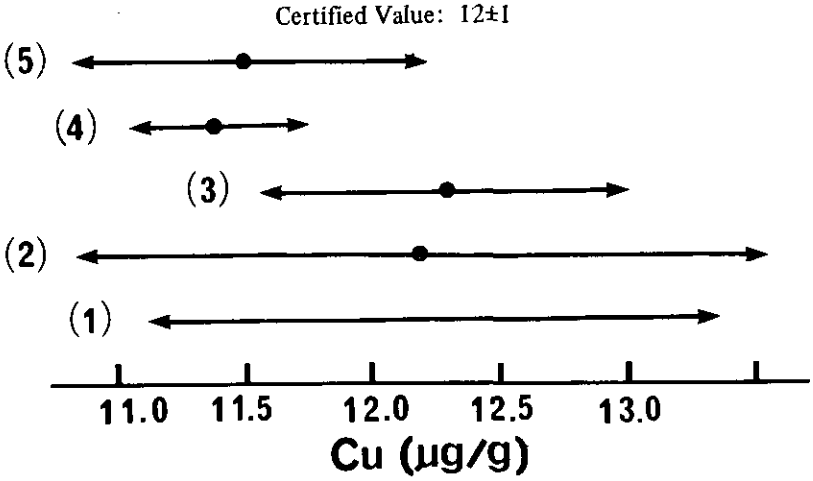

Fig. 1. Summary of mean values and confidence intervals for copper by various analytical techniques.

(1) Entire range of values reported.
(2) Mean ±2-sigma (all values).
(3) 95% confidence interval for the mean (AAS).
(4) 95% confidence interval for the mean (IDMS).
(5) 95% confidence interval for the mean (ICP).

## Table V

Certified and Reference Values for Pepperbush, NIES SRM No. 1

| Certified Values | |
|---|---|
| Element | Content* |
| MAJOR CONSTITUENTS | (Wt. Percent) |
| Potassium | 1.51±0.06 |
| Calcium | 1.38±0.07 |
| MINOR CONSTITUENTS | (Wt. Percent) |
| Magnesium | 0.408±0.020 |
| Manganese | 0.203±0.017 |
| TRACE CONSTITUENTS | (μg/g) |
| Zinc | 340±20 |
| Iron | 205±17 |
| Barium | 165±10 |
| Sodium | 106±13 |
| Rubidium | 75±4 |
| Strontium | 36±4 |
| Cobalt | 23±3 |
| Copper | 12±1 |
| Nickel | 8.7±0.6 |
| Cadmium | 6.7±0.5 |
| Lead | 5.5±0.8 |
| Arsenic | 2.3±0.3 |

| Reference Values | |
|---|---|
| | (μg/g) |
| Phosphorus | (1100) |
| Chromium | (1.3) |
| Cesium | (1.2) |
| Thallium | (0.13) |
| Mercury | (0.056) |

* Dry weight: 1. Dry in an air-oven at 85 ℃ for 4 hrs, or,
2. Dry in a desiccator over silica gel for 10 days.

## References

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We are indebted to the collaborating laboratories for their co-operation and particularly to members of the research group on SRMs under the chairmanship of Professor Toshiyasu Kiba (Kanazawa Institute of Technology). Without the endeavour of the participating laboratories the certified values for Pepperbush could not have been issued. The assistance and co-operation afforded by the National Bureau of Standards, U.S.A. also is gratefully acknowledged. Special thanks are due to Dr. C.W. McLeod for correction of English and Mrs Y. McLeod who undertook offset typing of the original manuscripts.

# 環境標準試料「リョウブ」の調製，分析および保証値

計測技術部　岡本研作（編）

環境問題の深刻化に伴い，現在の環境の質を評価し，また将来の変化をは握するために，おびただしい数の環境試料の分析が行われている。環境試料という性質上，分析対象は大気，水，土壌，生物等地球上に存在するすべての種類の物質を含んでおり，またそれらは気体，液体，固体と各種の形態をもっている。

環境試料の特徴として，(1)対象となる物質の種類，形態が多い。(2)試料の組成が極めて複雑であり，かつ試料ごとに大きく異なる。(3)測定対象となる物質の濃度が，微量から高濃度まで広範囲にわたっている。(4)測定対象となる物質は，試料中で様々な形態で存在している。(5)物理的，化学的変化や微生物による変性を受けやすく，サンプリング，試料調製，保存がむずかしい。(6)試料の均一性が乏しい。等があげられる。これらの特徴は，環境試料の分析を一層困難なものにしている。

分析値が一致しないことが常に問題にされ，分析精度の管理，向上のために同一試料を用いたクロスチェックの必要性が認識され，特に近年，環境試料を用いたクロスチェックが各方面で実施されている。クロスチェックは多くの場合，試料処理法，測定方法を同一にして行われるが，いまだに各機関の間で分析値のばらつきが見られており，もし各機関ごとに独自の分析方法を用いれば，このばらつきがもっと大きくなることは容易に想像できよう。

環境試料の分析において，現在，最も要求されていることは，データの質（言いかえれば正確さと精度）と言っても良いであろう。例えば以前に報告されている分析値の信頼性が乏しいため，測定対象物質の経時変化が追跡できないという問題が起こっているし，また正確な分析法が確立されていないために，ある種の環境問題にどう対処するかという行政的な判断ができない場合もある。

分析値の信頼性を高めるための方法として一般的に次の二つが考えられる。一つは，試料と同時に標準試料を分析して，分析値の正確さをチェックする方法であり，一つは正確さの証明された標準分析法を確立することである。当計測技術部において，分析化学的な基礎研究に加えて，環境試料の分析の精度向上に資するため，「環境標準試料の作製と評価に関する研究」が経常研究の一つとして昭和５２年度より開始されたことは，以上のような背景を反映している。本研究報告書は，国立公害研究所で最初に完成された環境標準試料「リョウブ」（Pepperbush，NIES №１）について，その調製法，分析法および保証値を中心として記載してあり，８章から成る。

なお，国立公害研究所で現在使用できる分析方法は限られているので，標準試料の保証値を決定するためには，様々な分析技術をもつ機関と協同で分析することが不可欠である。国

立公害研究所では，このような主旨から，分析化学に長年従事されている20名の方々に，リョウブ試料の分析依頼を行い，分析結果を提供していただいた。

第7章を執筆していただいた室住正世教授（室蘭工業大学）もその一人であり，特に同位体希釈質量分析法が，絶対分析法としてこの研究に極めて重要であることから，分析結果を更に詳しくして第7章として記載されている。

## 第1章　環境分析における標準試料

## Standard Reference Materials in Environmental Analysis

計測技術部　安部喜也

本章は，この報告書における序編として，環境分析の目的と現状，環境試料の特徴，標準試料の用途，標準試料たるべき必要条件，および国立公害研究所における標準試料プログラムの現状について述べる。

標準試料とは，その物質中の測定対象となる特性が正確に定量されかつ保証されている物質を意味する。例えば，現在我々が手がけている元素組成に関する標準試料であれば，その中の各元素の含有量が保証されている物質である。標準試料の用途として，(1)測定システムを校正するための標準として使われる。すなわち，標準試料を分析することにより，用いた分析方法の正確さを検定することができる。(2)新しい分析方法を開発する過程で，標準試料を使ってその方法の正確さと精密さが判定できるので，新しい分析方法の信頼性を確立するために役立つ。(3)標準試料を定期的に分析することにより，長期間の分析値の精度管理一特に現場分析において一を行うことができる。等が考えられる。

標準試料たるべき必要条件として，(1)測定対象となる特性が，正確に定量されかつ保証されていること，(2)社会的要求の強い試料であること，(3)均一かつ保存性の良い試料であること，(4)適当な濃度の元素を含むこと，(5)10年程度供給できるだけの大量の試料があること，(6)材料の大量入手が可能で，安価であり，かつ輸送が安全であること，等があげられる。

地球化学の分野では，1955年に米国地質調査所（USGS）において岩石標準試料G－1，W－1が作られ，岩石分析の精度および正確さの向上に大きな役割を果した。日本においても，地質調査所の安藤氏らにより，JG－1，JB－1といった優れた岩石標準試料が作製されている。一方，生物や環境試料などの，いわゆる"Soft"な物質についての研究の歴史は浅く，1960年代はじめの，イギリスのBowenによるKale powder（キャベツの粉末）が最初である。標準試料を現在最も組織的に配布しているNBS（National Bureau of Standards，米国標準局）においても，1970年代に入って環境標準試料の作製に重点をおき，Orchard leaves（果樹葉），Bovine liver（子牛肝臓）は，

今や分析の精度管理に欠かせないものである。この数年間，環境標準試料の数は急速に増えつつあるが，このように標準試料は社会的要求と密接に関係している。

標準試料中の保証すべき特性には様々な分類があるが，現在，国立公害研で作製している標準試料は，元素含有量に関するものである。ＮＩＥＳ№１「リョウブ」に続いて，№２「池底質試料」（Pond Sediment）についてはすでに分析がほぼ終了し，現在，保証値を決定する作業を進めている。№３「クロレラ」は代表的な緑藻の一つとして，すでに調製が終わり，試料の均一性のテストおよび分析が進行中である。人体試料は環境試料の中でも重も重要なことは疑いのないところである。№４「人血清」が調製され，№５「頭髪試料」の作製が進められているのは，このような理由による。リョウブ試料に続いて，これらの試料についても，国立公害研究所研究報告としてまとめられる予定である。

## 第２章　リョウブの特徴

## Nature of Pepperbush Plant

計測技術部　岡本研作

本章では，リョウブの重金属蓄積植物としての特異性，リョウブ中に蓄積された重金属の存在状態およびリョウブに対するコバルトの必須性の実験について述べる。リョウブが葉の中にコバルトを蓄積することは，すでに知られていたが，リョウブはこの他に亜鉛，マンガン，ニッケル，カドミウムを選択的に蓄積し，結果的にリョウブの元素組成は他の植物標準試料とは大きく異なっている。このことが，リョウブを新しい標準試料の材料として選定した一つの理由である。

リョウブ中の重金属の存在状態をゲルクロマトグラフィー等で調べた結果，コバルト，マンガン，カドミウムは低分子物質として存在し，一方，銅，亜鉛の一部は高分子と結合して存在する。銅については，生化学的手法を用いて銅結合高分子の精製を行い，単離した銅タンパクの分子量，吸収スペクトル，金属含量の測定，アミノ酸分析を行い，リョウブ中の単離された銅タンパクはプラストシアニンであることが判明した。この実験は，生化学的な見地からの研究の他に，元素の存在状態をも含めた形での標準試料を作製するための基礎実験でもある。

また，さし木より生育させたリョウブ苗を用いて，水耕法によりコバルトの必須性を調べた。本実験ではコバルトの必須性は証明されなかったが，水耕液に加えたコバルトの化学形態（硫酸コバルトまたはビタミン$B_{12}$）の違いにより，コバルトの取り込み量が大きく異なること，同時に他の重金属（鉄，亜鉛，マンガン等）の吸収もコバルト濃度とその化学形態に大きく影響されること，が判明した。このことはリョウブを人工的に栽培することにより，

金属の濃度の異なった一連のリョウブ標準試料を作製できる可能性を示している。

## 第３章　リョウブ標準試料の調製

## Preparation of Perpperbush

計測技術部　岡本研作

本章ではリョウブ標準試料の調製法について述べる。リョウブ標準試料の調製にあたっては，標準試料ができる限り環境中での状態と同じであり，環境試料を代表できるように，材料の迅速な処理および調製操作における容器等からの汚染の防止を心がけた。栃木県足尾町より採取したリョウブ葉（約100kg）を蒸留水で洗浄後，８０℃の通風乾燥器で２４時間乾燥した。乾燥リョウブ葉はアルミナボールミルを用いて粉砕し，ナイロンふるいを通して８０メッシュ以下の部分を集め，二分器を，繰り返し通して均一化した。混合した試料を1,150本のガラスびんに充てんし，（各１４ｇ），この中から３８試料をランダムに抜き取って，亜鉛，鉄，マンガンの含量を定量して試料の均一性を調べた結果，リョウブ試料中の亜鉛，鉄，マンガン含量の変動係数は，測定方法（原子吸光法）自身の繰り返し精度とほぼ同じであり，上記のようにして調製されたリョウブ試料は標準試料として十分に均一であることが判明した。

## 第４章　原子吸光法および炎光分析法によるリョウブの分析

## Analysis of Pepperbush by Atomic Absorption and Flame Emission Spectrometry

計測技術部　岡本研作

本章では，最初に原子吸光法の特長および現状について述べ，次にリョウブ試料の分解法，測定条件，分析結果について記載してある。定量を行った大部分の元素については，テフロンビーカー中で$HNO_3-HF-HClO_4$を用いて分解を行った後，フレーム原子吸光法により測定した。鉛については，図示した閉鎖系の分解装置中で$HNO_3-H_2O_2$を用いて分解を行い，ノンフレーム原子吸光法により定量した。

同時に炎光分析法を用いてアルカリ金属，アルカリ土類金属の定量を行い，その分析値を示す。

## 第5章　プラズマ発光分析法によるリョウブの元素分析

## Elemental Analysis of Pepperbush by ICP Emission Spectrometry

計測技術部　C. W. McLeod, 古田直紀, 西川雅高

プラズマ発光分析法は，この数年来急速に進歩している比較的新しい多元素同時分析法であり，リョウブ中の金属元素の分析に用いられた。本章では，プラズマ発光分析法の原理，最適条件の設定，およびリョウブ試料の分析について述べる。

本法の特長として，①金属および非金属の定量が行えること　②最も感度の良い最適条件が，ほとんどの元素に対して共通であること　③多元素同時分析が行えること，④検量線の直線領域が広く，試料の希釈を必要としないこと，⑤化学干渉が比較的少ないことなどがあげられる。

リョウブ試料の分析には二つのタイプの装置（直続方式とスルースキャン方式）が用いられた。直続方式は，光電子倍増管をそれぞれの元素の分析線の位置に並べた多元素同時分析用であり，広く用いられている。まず各元素に対するプラズマの最適条件を求めるために，三つのパラメーター①RF出力，②トーチ位置，③試料吸引速度を変えて実験を行った。その結果，元素によって若干の差は見られるが最適条件として①RF出力1.1kW②トーチ位置19mm，③試料吸引速度1.0mℓ／minが求められた。直続式プラズマ発光分析装置による各元素ごとの検出限界および最適条件下で定量したリョウブ試料の分析値を示す。

スルースキャン型は，必要ないくつかの波長を逐次掃引する方式であり，検出器としてSITを用いている。特に環境試料のように複雑な組成をもつ試料に対して，本方式は，バックグランド補正に大きな威力を発揮する。リョウブ分解溶液中の元素のスペクトルおよび本装置による定量値を示す。

## 第6章　エネルギー分散けい光X線分析法によるリョウブの分析

## Analysis of Pepperbush by Energy－Dispersive X－ray Fluorescence Spectrometry

水質土壌環境部　高松武次郎

けい光X線分析法は，試料にX線を照射し出てくる元素固有の特性X線を検出し，その強度から元素の定量を行う方法である。本法の特長として，①非破壊分析法であるので，試料調製が簡単であり，同一試料を繰り返し測定することができる。②Na以上の元素が測定可能で，その検出感度はおおむね原子番号から推測できる。③主成分および微量成分を同時に定量することができる。④スペクトルの干渉が比較的少ない。⑤　スペクトルは，一般的に，元素の物理的状態や化学形態の影響を受けない。⑥特に生体試料の場合，主成分は軽元素なのでマトリックス干渉が比較的少ない。などがあげられる。

けい光X線分析法は，エネルギー分散型と波長分散型に分けられるが，ここで用いたエネルギー分散型は後者に比べて，①多元素同時分析が行えること，②検出感度が高いこと，③スペクトルがコンピューター処理に適していること，④X線管の出力が小さいので，装置の小型化，安定性，運転経費の点で優れている，等の利点をもっている。

リョウブ試料の分析には，セルロースに既知量の目的元素を添加し，内部標準としてＣｓとＳｅを添加した合成標準試料をスタンダードとして用いた。リョウブのけい光X線スペクトルおよび上記合成標準試料とＮＢＳ標準試料を用いて作成した検量線を示す。検量線は良い直線性を示し，これを用いて定量したリョウブの分析値を示す。

## 第７章　同位体希釈質量分析法によるリョウブ中のアルカリ金属，アルカリ土類金属，重金属の定量

## Determination of Alkalies, Alkaline Earths and Some Heavy Metals in Pepperbush by Isotope Dilution Mass Spectrometry

室蘭工業大学　室住正世・中村精次・五十嵐龍志・菅　和哉・吉田勝美

同位体希釈質量分析法は，その原理によりみて，最も正確さの高い分析法の一つであり，標準試料の分析のように正確さを要求される分野では不可欠な分析方法である。また，本法は超微量の金属元素の定量を行うので，試薬の精製，ふんい気からの汚染，スパイク相互間の不純物には十分注意して，汚染が起こらないようにしなければならない。同位体希釈質量分析法には，高純度試薬，高度のクリーンルーム，高価な装置，熟練した化学者が必要であり，国内で本法を行える研究機関は極めて限られている。本研究所報告に，同位体希釈質量分析法によるリョウブの分析に関する章を含めたのは，以上の理由による。

本章では，まず同位体希釈質量分析法の原理を述べ，クリーンルームの説明，高純度試薬およびスパイクの調製法を特に不純物のチェックに重点をおいて述べている。試薬中の不純物およびスパイク相互間のコンタミネーションは，測定に全く問題とならない程少ない。

実際のリョウブ試料の分析に関しては，測定元素を（銅，カドミウム，タリウム，鉛），（銀），（ルビジウム，ストロンチウム，バリウム），（カリウム，カルシウム，マグネシウム）の四つのグループに分けて，各グループごとに試料の分解，調製法，スパイクの種類と濃度，およびリョウブの分析値について詳しく記載してある。

## 第8章　リョウブの保証値

## The certification of Pepperbush

計測技術部　岡本研作

標準試料は，標準となるべき特性が確立されて初めて，標準試料たりうることは明らかで

である。標準試料の保証値を決定することは決して簡単なことではないが，まず最初に様々な分析方法を用いて試料の分析が行われなければならない。この際，最も重要なことは，分析値の正確さであり，できる限り真値に近いことが望ましい。真値とは本当は，“神のみぞ知る”値であって，知り得ないのであるから，いかに真値に近い値を得て，保証値と呼ぶことができるかということになる。

国立公害研究所で現在使用できる分析方法は限られているので，様々な分析技術をもつ機関と協同で，リョウブ試料の分析を行った。次の１２の独立した分析方法がリョウブの分析に用いられた。(1)原子吸光法，(2)炎光分析法，(3)プラズマ発光分析法，(4)けい光Ｘ線分析法，(5)同位体希釈質量分析法，(6)中性子放射化分析法，(7)光量子放射化分析法，(8)同位体希釈不足当量法，(9)吸光光度法，（10）けい光光度法，（11）重量法，（12）ポテンシオメトリーである。

分析方法は，それが本来持つ正確さに応じて①絶対分析法，②比較分析法，③現場分析法の三つに評価，分類される。リョウブ標準試料の保証値の決定に当たっては，原則として，①絶対分析法による二つ以上の独立した分析値があること，または②二つ以上の独立した比較分析法による分析値があること，を基準としている。しかし，①の絶対分析法のみによって保証値を決定することは，現状では不可能であるので，②の様式を用いてこれに絶対分析法による分析値も含めることになる。

保証値を決定したリョウブの１６元素に対して，少なくとも四つ以上の独立した分析方法がそれぞれの元素に対して用いられた。

銅を例として，保証値を決定した方法を示す。銅の分析値は１６あり，その内訳は，原子吸光法……８，プラズマ発光分析法……４，同位体希釈質量分析法……１，けい光Ｘ線法……２，中性子放射化分析法……１，である。１６の分析値の範囲は，１１.１～１３.４ ppm，その平均値±２倍の標準偏差は，１２.２±１.４ ppmとなる。原子吸光法に関しては，８機関の分析値について分散分析を行い，所内，所間の分散の推定を行った上で，平均値の９５％信頼区間を求めた。同位体希釈質量分析法，プラズマ発光分析法に関しては，一機関だけの分析値を用い，それぞれの平均値の９５％信頼区間を求めた。保証値を純統計学的に決定することは現状では不可能であるが，以上のデータをふまえ，個々の分析値の正確さについて詳細な検討を行った上で，銅の保証値（Certified Value）を１２±１ppmと決定した。保証値を決定した他の元素についても，同じ様式で保証値を決定した。

参考値（Reference Value）は，分析値は良く一致しているが，一種類のみの分析法によって定量されている元素であり，クロム，セシウム，水銀，タリウムがこれに相当する。また，リンについては吸光光度法，プラズマ発光分析法ともに，良く一致した値を示すが，試料の処理法，特に分解操作中での揮散の問題が残っているので参考値としてある。リョウブ試料の保証値（１６元素），参考値（５元素）を示す。

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**Report of Special Research Project The National Institute for Environmental Studies**

No. 1* Man Activity and Aquatic Environment – with special references to Lake Kasumigaura – Progress Report in 1966 (1977).

No. 2* Studies on evaluation and amelioration of air pollution by plants – Progress report in 1976-1977 (1978).

Starting with Report No.3, the new title for N.I.E.S. Reports was changed to:

**Research Report from The National Institute for Environmental Studies**

No. 3 A comparative study of adults and immature stages of nine Japanese species of the genus *Chironomus* (Diptera, Chironomidae) (1978).

No. 4* Smog chamber studies on photochemical reactions of hydrocarbon-nitrogen oxides system – Progress report in 1977 (1978).

No. 5* Studies on the photooxidation products of the alkylbenzene-nitrogen oxides system, and on their effects on Cultured Cells – Research report in 1976-1977 (1978).

No. 6* Man Activity and Aquatic Environment – with special references to Lake Kasumigaura – Progress Report in 1977-1978 (1979).

No. 7 A morphological study of adults and immature stages of 20 Japanese species of the family Chironomidae (Diptera) (1979).

No. 8* Studies on the biological effects of single and combined exposure of air pollutants – Research report in 1977-1978 (1979).

No. 9* Smog chamber studies on photochemical reactions of hydrocarbon-nitrogen oxides system – Progress report in 1978 (1979).

No. 10* Studies on evaluation and amelioration of air pollution by plants – Progress report in 1976-1978 (1979).

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No. 18 Preparation, Analysis and Certification of PEPPERBUSH Standard Reference Material (1980).

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