Japanese Tea Leaves: A Possible Biological Standard Reference Material

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Japanese Tea Leaves, prepared by pulverizing with an agate ball mill and sieving with a Saran fiber sieve (50 mesh) were assessed as a possible biological standard reference material for elemental analysis. The metal content of the tea leaves was determined independently at two laboratories using atomic absorption and flame emission spectrometry. Neutron activation analysis was also performed to determine the content (21 elements) of Tea Leaves. For some elements the result from the various methods were compared. The characteristics of Tea Leaves are discussed and the elemental composition is compared to that of Orchard Leaves (NBS SRM, 1571). The most significant characteristic of Tea Leaves was the high manganese content.

The need for standard reference materials in elemental analysis is steadily increasing, due primarily to the ever-increasing demand for elemental data in many scientific fields.

Standard reference materials were first prepared in 1904 by NBS.¹⁾ They were metal samples such as cast iron in chip form. United States Geological Survey (USGS) prepared in 1955 standard rock samples, G 1 and W 1, which were distributed to analytical laboratories throughout the world.²⁾

After 1960 many studies concerned with standard reference materials have taken place. Bowen's³⁾ study on "kale powder" began in 1960 and in this study, 42 elements were determined at many laboratories using 11 different analytical methods. Vallee⁴⁾ has studied the metal content of bone ash standard sample. Jones,⁵⁾ a member of Bowen's group has studied eight grass samples by direct-reading emission spectrometry. The present authors have determined the metal content of these grass samples by atomic absorption spectrometry.⁶⁾ Sideman's⁷⁾ work on serum samples was concerned only with Ca and involved participation by 31 laboratories. Yamagata's⁸⁾ work on Cd in rice was brought about from environmental Cd contamination resulting in abnormally high Cd level in rice production. The work by NBS research group¹,⁹⁾ has been the most detailed and of the most practical use.

Recently, trace element analyses of various types of biological and/or environmental samples have been performed so extensively that it has become necessary to prepare new biological standard materials. As part of a co-operative study with NBS for the development of new biological standard materials, Japanese Tea Leaves were investigated as a possible standard reference materials. The sample was prepared on a small scale and analysis using two analytical methods, atomic absorption spectrometry and neutron activation analysis, was performed at the University of Tokyo and the National Institute for Environmental Studies.

Experimental and Results

Preparation of Tea Leaves. Three kinds of tea leaves were obtained from National Research Institute for Tea. They were (1) "Sen-cha", a middle grade green tea(Tea B), (2) old tea leaves which are not suitable for drinking(Tea C) and (3) powdered tea leaves(Tea D). The weight of the original tea samples was about 1 kg.

TABLE 1. METAL DISTRIBUTION IN TEA LEAVES (μg/g)

Sample	K	Ca	Mg	Mn	Fe	Zn
B Powder	23300	2320	1770	610	142	66
Part retained under sieve	27000	3260	1840	660	104	59
C Powder	10700	6870	1350	2650	241	19
Part retained under sieve	10200	7130	1300	2310	160	16

Tea samples B and C were pulverized with an agate ball mill and passed through a sieve made of Saran(50 mesh). The rigid parts of the leaves such as leafstalks were retained by the sieve and for both tea samples(B and C) the amount retained was approximately 100 g. Elemental analysis of both parts indicated that more Ca and less Fe were contained in the unpowdered part retained on the sieve. The powdered part which passed through the sieve was used for the further elemental analysis.

Tea D, a homogenized powder was analysed directly.

Preservation of the Samples. The powdered samples were stored in a desiccator containing silica gel and weighed periodically to determine whether the weight changed during storage. Standard materials should be preserved without any change for long periods. The results, summarized in Table 2, indicate that the dry weight did not alter significantly within 6 months.

TABLE 2. CHANGE OF WATER CONTENT DURING STORAGE OF TEA POWDER IN DESICCATOR (Silica Gel)

Sample	Water content (%)	
	Nov., 1975	May, 1976
Tea B	5.4	6.0
Tea C	7.2	6.6
Tea D	7.4	5.9

Water content was measured after 24 h drying at 90 °C.

Homogeniety of the Sample. To determine whether the powdered samples were homogeneous, the Fe content of 18 randomlly selected portions (0.5 g) of Tea C was determined

Table 3. Fe content in tea powder C

	Fe(μg/g)	
Bottle 1	262	
	240	
	249	av. 250
Bottle 2	246	
	228	
	237	av. 237
Bottle 3	253	
	241	
	235	av. 243
Bottle 4	244	
	240	
	236	av. 240
Bottle 5	243	
	247	
	234	av. 241
Bottle 6	241	
	250	
	250	av. 247

average 243,
range 228—262,
c.v. 3.5%.

by atomic absorption spectrometry. Portions (0.5 g) of sample (C) were taken from three parts of every six bottles of Tea C. The tea powder was contained in six sample bottles and each sample bottle was sub-divided in three equal parts before the portions were selected. The results of the analysis of the 18 sub samples are shown in Table 3, and indicate that the homogeniety of the tea sample is similar to that of Orchard Leaves.

Table 4. Experimental conditions for atomic absorption measurement

	National Institute for Environ. Studies	The University of Tokyo
Desiccation	105 °C upto constant	90 °C, 24 h
Sample amount	1 g	0.5 g
Ashing Wet:	HNO_3 (4 ml) $+H_2O_2$ (3 ml) 100 ml flask with cooler and trap	HNO_3 (4 ml) $+H_2O_2$ (3 ml) 50 ml Kjeldahl flask
Dry:	450 °C 15 h	
Atomic absorption		
Instrument Flame:	Hitachi 508	Jarrell-Ash AAI-Mark 2, Seiko SAS 721
Flameless:	Perkin-Elmer 503 +HGA 2000	Varian-Techtron AA 1000+CRA 63
Standard solution,	Metal in 0.1 N HNO_3	Metal in Matrix (K 200, Ca 50, Mg 20, P 20, Na 17, Al 10, Mn 10 μg/ml)

Determination of Mn, Fe, Zn, and Pb by Atomic Absorption Spectrometry. Atomic absorption determination of Mn, Fe, Zn, and Pb was performed by two laboratories, independently. The experimental conditions employed by the two laboratories are shown in Table 4. In the work, different sample pretreatments were adopted and different standard solutions were used. At the National Institute for Environmental Studies, standard metal ion solution containing nitric acid(0.1 M) was used while at the University of Tokyo, a a matrix solution which contained the particular metal ion as well as other major elements in the tea leaves was used. At the University of Tokyo, the optimum conditions of flame atomic absorption measurement for each element was decided using the response-surface plot method, details of which have been described elsewhere.[10)]

Table 5. Fe, Mn and Zn contents in tea leaf samples and orchard leaves

		National Inst for Environ. Studies		The Univ. of Tokyo	(Certified value)
Fe	Tea B	135[a)]	137[b)]	142	
	Tea C	242	226	241	
	Tea D	375	438	423	
	Orchard leaves	268	239	281	300±20
Mn	Tea B	626	620	605	
	Tea C	2760	2690	2650	
	Tea D	977	984	1010	
	Orchard leaves	91	91	95	91±4
Zn	Tea B	59	61	66	
	Tea C	20	19	19	
	Tea D	95	85	90	
	Orchard leaves	25	29	32	25±3

(μg/g) a) Wet ashing. b) Dry ashing.

Table 6. Pb contents in tea leaf samples and orchard leaves

	National Inst. for Environ. Studies		The Univ. of Tokyo	(Certified value)
Tea B	2.9[a)]	3.4[b)]	1.8	
Tea C	5.6	5.5	2.9	
Tea D	4.0	3.4	2.0	
Orchard Leaves	43.3	16.1		45±3

(μg/g) a) Wet ashing. b) Dry ashing.

The results for Mn, Fe, and Zn are shown in Table 5 and indicate that the date for the two laboratories agree within 10% in most cases and that there is no significant difference between the data obtained by wet ashing and those by dry ashing. Concentrations of Pb determined by carbon rod furnace atomic absorption spectrometry at the two laboratories do not agree. This is shown in Table 6. This discrepancy may be caused by the difference in the background correction system adopted by the two laboratories and/or by the chemical interference from the matrix elements. This is presently under investigation.

Neutron Activation Analysis. In addition to atomic absorption and flame emission spectrometry mentioned above, 21 elements including Mn, Fe, Zn, K, Ca, and Mg were determined by conventional non-destructive neutron activation analysis. Neutron irradiation were carried out in 100 KW TRIGA Mark II Reactor of Rikkyo University. Each tea sample (almost 100 mg) was irradiated under a thermal neutron flux of 5×10^{11} for 5 min. A 4048 channel pulse height analyzer coupled with a Ge(Li) detector was used to detect the γ-rays which were emitted from the radioactive nuclides. Al, V, Cu, Ca, and Mg were determined after 1 to 2 min irradiation, while Cl, Mn, K, and Na were determined after 1 to 2 h irradiation. The samples were further irradiated under a thermal neutron flux of 5×10^{11} n cm^{-1} s^{-1} for 30 h and As, Br, La, Sm, Ba, Rb, and Cr were determined after 5 to 6 days. Finally Fe, Sb, Se, Zn, and Co were determined after about 100 days. In this work,

Table 7. Elementary composition of tea leaf samples and orchard leaves (μg/g)

	Tea B[a]	Tea C[a]	Tea D[a]	Orchard Leaves	
				Found[a]	Certified
K	24000±8000	11000±4000	22000±7000	13000±3000	14700±300
Ca	2500±400	4200±900	1900±400	20000±500	20900±300
Mg	1300±400	5100±2400	1000±600	7100±1100	6200±200
Cl	1700±100	1200±100	1100±100	910±50	(700)
Mn	530±4	2300±10	890±8	102±1	91±4
Al	420±10	2000±40	660±20	385±5	
Fe	280±150	360±210	440±230	290±40	300±20
Zn	55±8	13±3	65±9	20±2	25±3
Na	33±4	30±20	20±5	87±5	82±6
Rb	29±6	8±2	44±9	11±1	12±1
Cu	15±8	59±37	27±15	19±4	12±1
Br	13.0±0.2	25.0±0.3	7.8±0.1	11.7±0.4	(10)
Ba	2.3±0.8	5.1±0.8	1.3±0.8	46±9	
Cr	1.1±0.3	1.2±0.3	0.8±0.2	2.8±0.3	(2.3)
Se	1.0±0.2	1.8±0.3	1.2±0.2		
As	0.42±0.23	0.20±0.05	0.31±0.12		10±1
V	0.2±0.1		0.4±0.1	0.82±0.07	
La	0.19±0.01	1.60±0.03	0.26±0.01	1.3±0.1	
Sb	0.15±0.06	0.14±0.08	0.11±0.06	3.0±0.1	
Co	0.10±0.02	0.16±0.02	0.18±0.02	0.19±0.04	(0.2)
Sm	0.023±0.002	0.23±0.01	0.032±0.003	0.13±0.01	

a) Determined by neutron activation analysis. Errors correspond to the statistical errors on counting radioactivities.

elemental standards and Orchard Leaves were irradiated together with the tea samples.

The results for the three tea leaf samples (B, C, D) are shown in Table 7, together with those for Orchard Leaves. Errors cited in this table correspond to the statistical errors on counting radioactivities. The elemental composition of Orchard Leaves determined in this work compares favorably with that reported by NBS[9]. The results for V, Cu, Na, and and K which were determined at short irradiation time (5 min) have large errors. This was due to the relatively large amount of Mn in the tea leaves which resulted in the over-production of ^{56}Mn at long irradiation time.

Discussion

Of the elements determined by neutron activation analysis, Mn, Fe, Mg, and Zn were also determined by atomic absorption spectrometry and K and Ca by flame emission spectrometry. A comparison of the data for Zn, Mn, and Ca is shown in Figs. 1—3, respectively. These results indicate that the metal

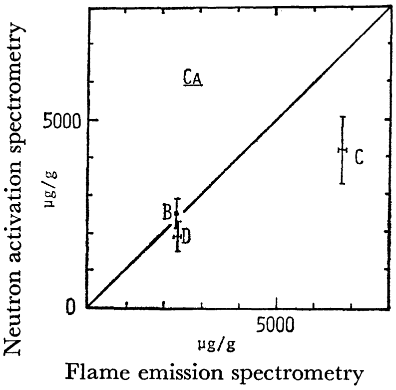

Fig. 1. Comparison of calcium content determined by activation analysis and flame emission spectrometry.

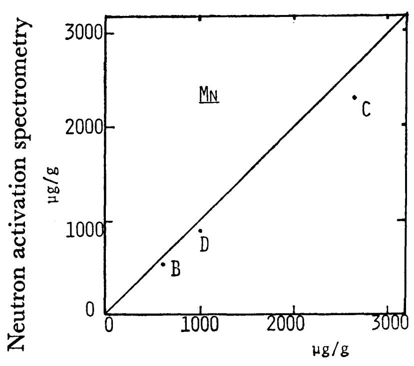

Fig. 2. Comparison of manganese content determined by activation analysis and atomic absorption spectrometry.

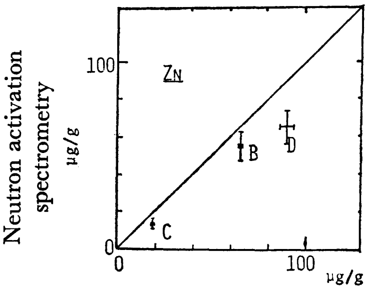

Fig. 3. Comparison of zinc content determined by activation analysis and atomic absorption spectrometry.

contents determined by activation analysis are slightly lower than those for emission or absorption spectrometry. The results also agree with the comment of Bowen:[3] "the differences (in analytical results) between techniques were usually larger than the differences between individual laboratories in which the same technique was used." In our case, the difference in analytical data for the two laboratories by the same technique (atomic absorption spectrometry) is smaller than that by the different techniques (atomic absorption or flame emission spectrometry and neutron activation analysis).

To decide whether the tea leaf samples are suitable as a new biological reference material, the results obtained must be assessed. The prerequisites for a standard reference material might be listed as follows:

1. Availability in large amount.
2. Availability at low cost.
3. Preservation for a long period.
4. Safety for transportation.
6. Appropriate elemental composition.

Point 1 and 2 are met since there is a supply of tea leaves at low cost. Tea leaf samples were shown to be preserved for at least six months. In the case of biological samples, the alteration caused by activities of microbes should be considered as well as the chemical alteration. Irradiation of the samples by ^{60}Co, as was the case in preparing Orchard leaf samples at NBS,[11] is one of the most powerful techniques to prevent biological alteration. For tea leaf samples, a steaming procedure used in commercial processing of raw tea may be a suitable sterilization process.

One of the most important criteria for a standard reference material is homogeniety. The tea leaf samples prepared in this work were shown to be homogeneous (Fe determined in 18 randomly selected sub-samples of Tea C).

As a new biological standard, the elemental composition must also be characteristic compared with other similar kinds of standard materials produced in the past. In Figs. 4—6 and in Table 7, a comparison of the elemental composition between Orchard Leaves and Tea B, Tea C, and Tea D is shown.

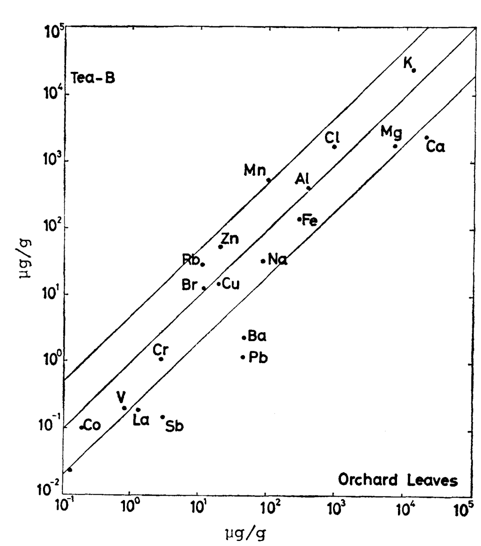

Fig. 4. Elementary compositions of Tea B and Orchard Leaves.

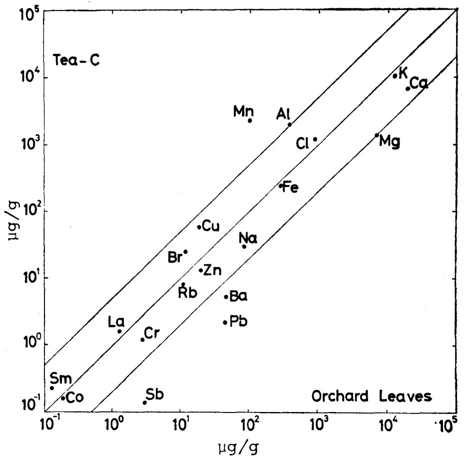

Fig. 5. Elementary compositions of Tea C and Orchard Leaves.

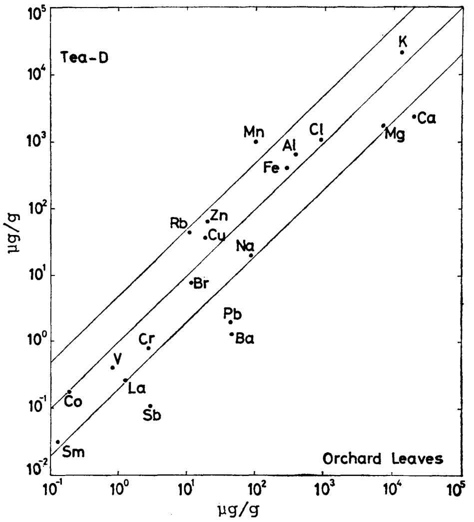

Fig. 6. Elementary compositions of Tea D and Orchard Leaves.

The characteristic features of the elemental compositions are summarized as follows:

1. The Mn content of the tea leaf samples is much higher than for Orchard Leaves.
2. The Ca and Ba contents are lower in the tea leaf samples.
3. As for the other elements, the content in the tea leaf samples is similar to that of Orchard Leaves.

Growth of the tea plant in acid soil may reflect the high content of Mn in the tea leaf samples. The high level of Mn might make tea leaf samples to be preferred as a standard reference material when acid-resistant plants such as rice, are analysed. In addition, it is necessary to eliminate contamination during the preparation procedure even if the homogeniety of the contaminant elements is certified. For example, the Pb content in Orchard Leaves is relatively greater than for most other edible plant products. This suggests that the Pb levels in Orchard Leaves may partly arise from contamination from sources such as pesticides. The low recovery of Pb in Orchard Leaves by ashing compared to that for tea leaf samples may indicate that a certain part of Pb in Orchard Leaves is in an inorganic form which may behave differently from inert Pb at the ashing step (Table 6).

Practically, it would be difficult to eliminate contamination from foreign elements in the preparation process, but further work may resolve this and improve the sample product.

To summarize, tea leaf samples may be a candidate for a new biological standard reference material.

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