

Determination and Application of Trace Elements

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It is a well known fact that mineral plays an important role in the nutrition of animal and plant. The trace elements, which are a part of the mineral and exist in a very small amount in the living bodies, include not only essential elements but also harmful and useless elements.

In the researches up until now, physiological significance or essentiality for living body of such elements as cobalt, copper, iodine, iron, magnesium, manganese, molybdenum, selenium and zinc have been investigated. Recently, researches have been conducted on the harmful elements such as arsenic, cadmium, mercury and lead in relation to air, land and water pollution.

Initial studies are focused on the function and distribution of trace elements in the living bodies, and their quantitative determination in animal, plant, water, soil and air must be carried out for this purpose.

This report presents briefly the method of determination of trace minerals and some researches made by us.

Methods of determination

General chemical analysis was applied to quantitative determination of trace elements at the early stage and colorimetry was mostly used. This analytical method is very useful as a means to detect sensitivity and accuracy but it has a weak point in that it takes too much time and labor for the determination.

Recently instrumental analytical procedures

has remarkably progressed. Flame spectroscopy, polarography, emission spectroscopy, atomic absorption spectrometry, X-ray fluorescence method and activation analysis have been used for the trace analysis.

Each method has a speciality for the respective elements, so that it should be selected in accordance with the objective element. Of course, it is hoped that the methods have good detectable sensitivity, analytical accuracy and ability. When the sample is of a small amount, the method by which many kinds of element can be determined without interference using one sample preparation may be required.

At our institute, the emission spectrograph and the polarograph were installed in 1958. Using the emission spectrograph, a wide range of elements can be detected with respect to a small amount of samples and moreover the quantitative analysis is also possible under limited conditions.²⁾

For the determination of copper and zinc, the polarography is superior to the other methods in regard to sensitivity.¹⁾ Since 1968 when the atomic absorption spectrometer could be used, trace analysis has become easy in our institute.

Preparations of sample for determination

The trace elements exist generally in the forms of mixture or of compound with organic materials except when they are a part of the air or the water. Without some additional

treatment, every method of determination can scarcely be applied directly.

When the content of element measured is of smaller amount, more volume of material, labor and time are needed for the determination. Ashing procedure can not be omitted in the analytical process because of the concentration of trace component. The precision of analysis depends greatly upon those procedures.

For such elements at high temperature as cobalt, iron, manganese and molybdenum, which are unvolatile at high temperature, dry ashing of using the electric muffle at more than 450°C is employed. This dry ashing procedure can deal with a large amount of materials without any dangers and any contamination from chemical reagents.

In our laboratory, using atomic absorption the contents of copper, iron, manganese and zinc in pasture grass were measured after dry ashing at 350–700°C.

Satisfactory results were obtained when one gram of samples was ashed at 450°C for three hours and was treated with hydrofluoric acid.^{6,7)} In the case of blood or hair, dry ashing under 500°C is applied for the determination of copper and zinc.

When volatile elements such as arsenic, cadmium, mercury, iodine, lead and selenium were determined, wet ashing should be taken using such acids as nitric, sulfuric and perchloric.

In the low temperature ashing apparatus which has been sold since two or three years, the samples can be ashed at a low temperature of less than 200°C, utilizing the oxygen of its excited states. Thus, the recoveries of volatile trace elements are excellent. Low temperature ashing is suitable when some elements containing volatile elements are determined in one sample preparation.

Molybdenum poisoning of milch cow by aerosol contamination

In the spring of 1964, the outbreak of molybdenum poisoning of milch cow by aerosol

contamination from the factory occurred at the "A" district in Hyogo Prefecture.³⁾ The large amount of molybdenum was detected from forage plants supplied to cows by emission spectroscopy after dry ashing procedure.

As shown in Table 1, the molybdenum con-

Table 1. Molybdenum content in forage crops at "A" district in Hyogo Prefecture

Sample	No.	Mo ppm/dry basis
Rice straw	1	249.0
	2	19.0
	3	28.0
	4	167.0
	5	478.0
Cabbage	1	68.6
	2	194.4
	3	68.0
Oat soiling	1	89.0
	2	25.2
	3	91.3
Clover	1	224.0
	2	484.0
Turnip	1	55.3
	2	1.7
	3	64.0

tent of pasture grass in this district was abnormally high and was about one hundred times higher than that of pasture grass in other districts.

The milch cow in this area showed such characteristic clinical symptoms as distinctive diarrhea, anorexia, fading of coat color and abnormally high molybdenum content in the blood that it was apparent the animals suffered from molybdenum poisoning.

On the other hand, the molybdenum content in the soil and milk vetch (Renge) at every 200-m spot centering the factory was determined and the molybdenum content was increased centripetally.

From this fact, it was substantiated that the origin of contamination was the neighboring molybdenum refining factory. The

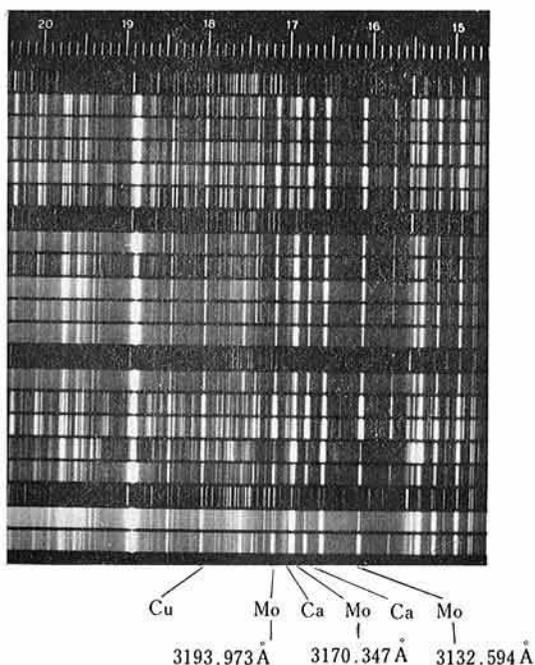


Fig. 1. Emission spectrographic photograph of forage crops

Fe lines are 1st, 7th, 13th and 19th from the above; rice straw—2nd-6th, cabbage—8th-10th, oat—11th, 12th & 14th, clover—15th-17th and turnip—18th, 20th and 21st.

molybdenum content of milk vetch obtained at places of one km from the factory was over 10 ppm per dry basis.

Trace elements of rice straw

By the use of the direct reader emission method, many kinds of elements can be determined in one sample preparation at the same time. But the fact is that it was difficult to determine simultaneously every element: for instance, cobalt which needs a large volume of samples because of very little trace contents and of level less than the limits of detectability; manganese which does not need a large volume because of a large amount of contents and of a high sensitivity.

Some 260 samples of the rice straws produced in the paddy field in 1966 were collected from almost all over Japan. Copper(Cu), chromium(Cr), iron(Fe), molybdenum, nickel(Ni), tin(Sn) and lead(Pb) were determined by emission spectrography, and zinc(Zn) was analyzed by polarography.⁴⁾ The results are shown in Fig. 2.

Although there was difference according to

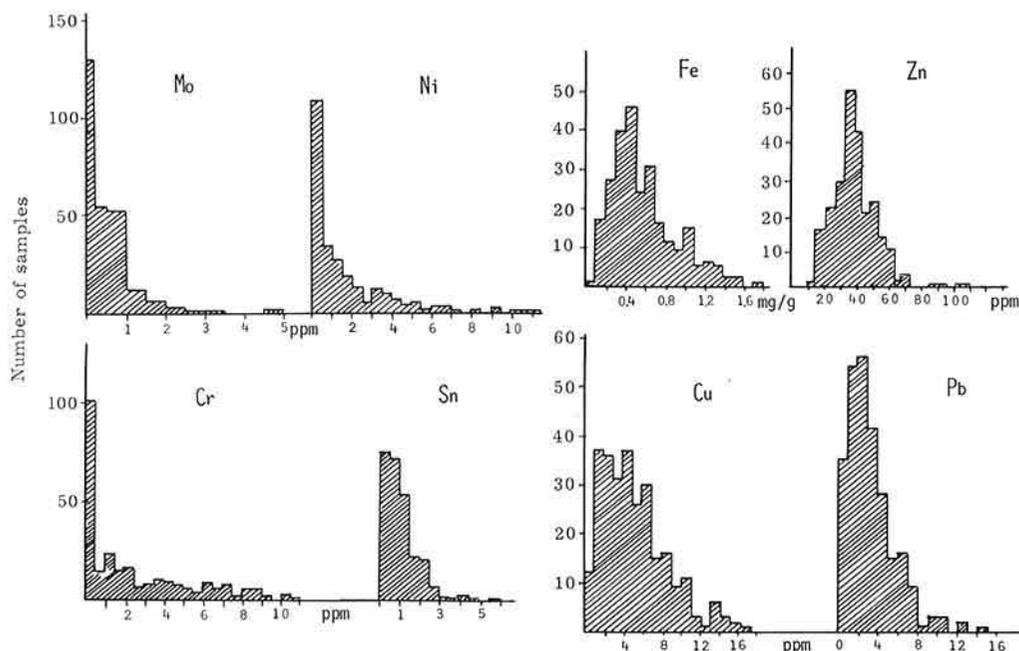


Fig. 2. Contents of trace elements in rice straw

the soils of paddy fields, fertilization and species, it seemed to represent the trace mineral contents of rice straw in Japan.

Trace elements of pasture grass

By the atomic absorption analysis several kinds of elements were possible to be determined on one gram of pasture.³⁾ One gram sample of dry pulverized material was ashed for three to four hours at 450°C., treated with hydrofluoric acid, and was made into 0.5 N hydrochloric acid solution.

Copper was determined on 10 times diluted solution, iron and manganese on 50 to 100 times solution and zinc on 10 to 100 times solution. Calcium and magnesium which are not trace elements, can easily be determined by diluting further the solution.

The results of determined on the above four elements in pasture cutting four times a year

at the same range are shown in Fig. 3.

In this range, copper and zinc contents of pasture were higher than that of the other range. The variance of copper content was little throughout the year. It may be considered that the large variance of iron and manganese contents was due to the variation of grassland consisting of grass and legume.

Copper, iron and zinc contents in blood serum

By the atomic absorption, contents of copper, iron and zinc were determined directly by quite low dilutions of serum with water. The average of copper, iron and zinc contents in the blood serum of 40 healthy cattle in the Tochigi Prefecture was $81 \pm 19.5 \gamma/\text{dl}$, $188 \pm 29.2 \gamma/\text{dl}$ and $83 \pm 13.3 \gamma/\text{dl}$ respectively. These values are considered as normal in cattle.

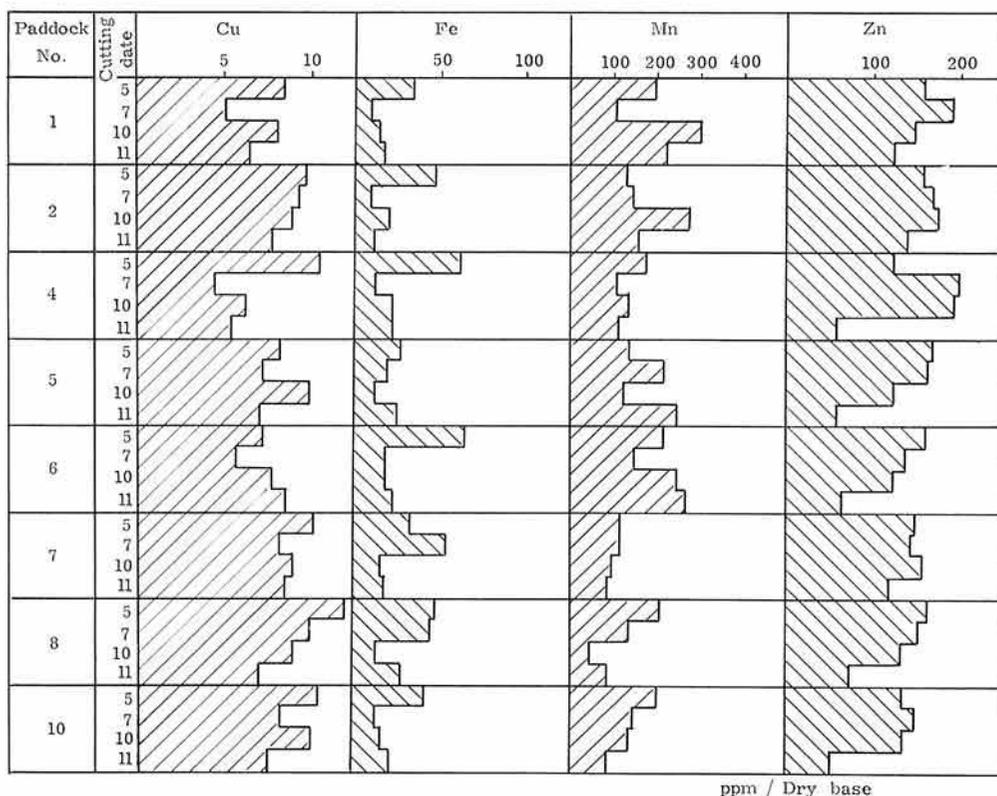


Fig. 3. Contents of trace elements in pasture grass

Cadmium (Cd) contamination and cattle feeding

Cadmium is so volatile an element that it should be ashed at low temperature. Atomic absorption was applied to samples after wet ashing with nitric and perchloric acids.

As shown in Table 2, the cadmium contents

Table 2. Cadmium content in liver and kidney of milch cow

	Cd pollution district		Control district
	No. 1	No. 2	
Liver	1.430	1.010	0.069±0.015*
Kidney	6.050	6.250	0.467±0.325*

*: average of 10 samples

The numeral shows the Cd ppm per wet tissues

of liver and kidney of milch cow scrapped at "A" district in Gumma Prefecture were found to be considerably higher than that at the Tokyo Meat Center.

As to the rice straws used as roughage, the cadmium contents from the paddy fields at "K" district in Tokyo that it considered to be polluted, were higher than that of rice straws produced in 1966 at the other non-polluted fields as shown in Table 3.

Table 3. Cadmium content in rice straw

Rice straw No.	"K" district Cd ppm	"cont." district Cd ppm	district place-name
1	3.650	0.095	Fukui
2	0.700	0.170	Shimane
3	0.850	0.303	Yamanashi
4	2.100	0.130	Wakayama
5	0.925	0.105	Iwate

Conclusion

Although at the present time the determi-

nation of trace elements has become more easily than before, it is still difficult to determine every kind of elements contained in the samples by only one method. Analytical methods may be chosen according to the elements of being measured and the condition of the laboratories.

A recent propagation of the atomic absorption analysis is very remarkable. A number of trace elements became to be easily determined by using this method.

The study of this field that has made little progress comparing with that of vitamin or hormone which is the same as trace in amount and have a striking physiological function will make rapid progress and moreover it may attract the attention of many people in the future in relation to environmental pollution.

References

- 1) Hayakawa, T.: Polarographic determination of zinc and cobalt in fodder. *Bull. Nat. Inst. Anim. Hlth.*, **39**, 121-129 (1960).
- 2) Hayakawa, T.: Emission spectrographic analysis of trace elements in plant materials. *Nat. Inst. Anim. Hlth. Quart.*, **1**, 53-62 (1961).
- 3) Ogura Y. et al.: Molybdenum poisoning of cattle due to aerosol contamination. *Bull. Nat. Inst. Anim. Hlth.*, **50**, 24-29 (1965).
- 4) Ogura Y. et al.: Trace elements of rice straw by emission spectroscopy, *Jap. J. Zootechn. Sci.*, **39**, Suppl. 87. (1969). [In Japanese. Summary of oral presentation.]
- 5) Ogura Y. & Yonemura T.: Variation of trace elements in pasture on two ranges, *Jap. J. Zootechn. Sci.*, **41**, Suppl. 32. (1970). [In Japanese. Summary of oral presentation.]
- 6) Ogura Y.: An ashing technique applied to plant materials for atomic absorption. *Nat. Inst. Anim. Hlth. Quart.*, **10**, 46-47 (1970).
- 7) Ogura Y.: Hydrofluoric acid treatment on pasture grass ashes for atomic absorption analysis, *Nat. Inst. Anim. Hlth. Quart.*, **10**, 171-172 (1970).