Inorganic Chemical Approaches to Pharmacognosy. VII.[1] X-Ray Fluorescence Spectrometric Studies on the Inorganic Constituents of Crude Drugs. (5). The Relationship between Inorganic Constituents of Plants and the Soils on Which They Are Grown

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Inorganic constituents of the leaves of several kinds of plants growing on different soil types were investigated using energy-dispersive X-ray fluorescence spectrometry.

The results can be summarized as follows: (1) Each plant exhibited a characteristic metals profile, even if they were grown on similar soil types. (2) As we would expect, the metals profile of the plant leaves depends on the inorganic constitution of the soil on which it is grown. However, the degree of the influence of the soil types on the metals profile of the plant differs according to the kind of inorganic element: Ca and Cu are almost independent of the soil types, but the Ti and Fe content is dependent upon the soil types. (3) Sr content of plant leaves is closely related to the ratio of Sr to Ca of the soil on which the plants are grown. (4) This information should be helpful in identifying the producing district or original plant of a crude drug by its metals profile.

Keywords energy-dispersive X-ray fluorescence spectrometry; multi-elemental analysis; inorganic constituent; soil type; strontium; plant leaf; producing district; metals profile; crude drug

In the course of our series of studies to elucidate the significance of inorganic constituents in crude drugs, we have reported on a convenient multi-elemental analysis method for crude drugs using energy-dispersive X-ray fluorescence spectrometry.[2] In addition, the analytical results for several kinds of crude drugs by this method have indicated that each crude drug has a characteristic metals profile which provides valuable information about the producing district or the original plant of a crude drug.[1,3,4] However, some problems still remain for the application of the metals profile to the identification of crude drugs: for instance, even the same kind of crude drug exhibits considerable variation in its metals profile. As one of the reasons for such variation of element content, the influence of the producing district, namely the inorganic constitution of the soil on which the original plant was grown is reasonably considered. Therefore, a knowledge of the relationship between the inorganic constitution of soil and the metals profile of the plant growing there is indispensable in understanding the inorganic constituents of a crude drug.

In this study, we paid attention to the different soil types (geological feature) between north and south of the Median Dislocation Line (MDL) in Tokushima Prefecture and collected several kinds of plants there together with the soils on which the plants were grown. The analytical results from these plant and soil samples by X-ray fluorescence spectrometry gave some valuable information for identifying the producing district (or original plant) of a crude drug.

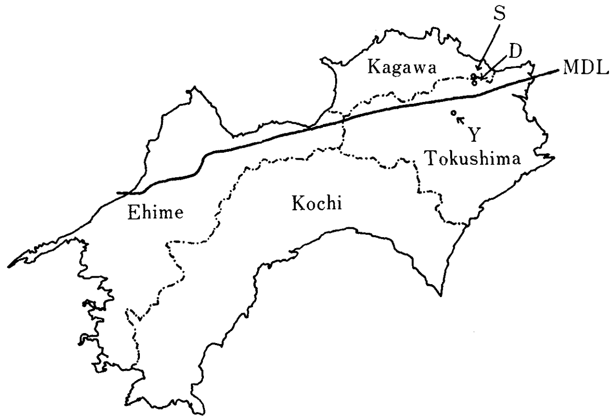

Fig. 1. Location Where Several Plants Were Collected

S, Shiratori-cho in Kagawa Prefecture; D, Donari-cho in Tokushima Prefecture; Y, Yamakawa-cho in Tokushima Prefecture.

Experimental

Apparatus X-Ray measurements were performed on a thin sample (48 mg/cm^2) with a Rigaku-Kevex energy-dispersive X-ray spectrometer (ultra-trace system), consisting of a molybdenum anode X-ray tube, secondary targets (Ti, Ge, Mo, and Gd) and a filter assembly used to generate bichromatic radiation, a 30 mm^2 × 3 mm Si(Li) detector, an X-ray amplifier, and a conventional multi-channel analyzer.

Materials Eight kinds of plant, *Petasites japonicus* (Compositae), *Peusedanum decursivum* (Umbelliferae), *Artemisia vulgaris* var. *indica* (Compositae), *Polygonum cuspidatum* (Polygonaceae), *Polystichum polyblepharum* (Polypodiaceae), *Boehmeria nivea* (Urticaceae), *Glechoma hederacea* (Labiatae), and *Taraxacum officinale* (Compositae), were collected at the north of MDL (*G. hederacea* and *T. officinale* were at Donari-cho, Tokushima Prefecture, and the others were at Shiratori-cho, Kagawa Prefecture), and at the south of MDL (all plants were at Yamakawa-cho, Tokushima Prefecture), together with the soils on which the plants were grown, in the middle of May, 1984 (see Fig. 1).

Procedures The leaves of each plant were air-dried, and powdered by use of a National coffee mill (MK-51), and/or agate mortar and pestle. 150 mg of each powdered sample was pressed into a 2.0 cm diameter pellet, followed by the X-ray fluorescence spectrometric experiments as described previously.[2] The soil samples were sieved to obtain narrow sieve fractions (less than 2000 μm), which were powdered by a Spex mixer mill (ball-type). The element concentrations of the resulting powders were analyzed by the Matsumoto and Fuwa's internal standard method using X-ray fluorescence spectrometry,[5] in which the known amounts of Sc, Ni, and Ge were added as internal standard substances.

Results and Discussion

The analytical results for eight kinds of plants collected at both areas, north and south of the MDL, are summarized in Table I. The metals profile depended considerably on the kinds of plants, even if they were grown on the same site. In the case of the plants growing on the southern locations, Cl content of *P. decursivum* and *P. cuspidatum* were near 0.4%, and that of *T. officinale* was more than 2.0%. The K content varied from 1.4% for *B. nivea* to 5.0% for *T.*

TABLE I. Analytical Results (% or ppm) for Some Plants by X-Ray Fluorescence Spectrometry

Element	*Petasites japonicus*		*Peucedanum decursivum*		*Artemisia vulgaris*		*Polygonum cuspidatum*	
	South ($n=3$)	North ($n=5$)	South ($n=3$)	North ($n=1$)	South ($n=4$)	North ($n=4$)	South ($n=3$)	North ($n=5$)
P	0.18 (1)%[a)]	400 (400)[a)]	0.31 (1)%	0.28%	270 (170)[a)]	700 (200)[a)]	0.23 (2)%	0.28 (4)%
S	0.65 (4)%	0.73 (9)%	0.52 (3)%	0.37%	0.28 (5)%	0.23 (1)%	0.23 (2)%	0.26 (2)%
Cl	1.0 (2)%	1.1 (2)%	0.40 (5)%	0.35%	1.1 (1)%	0.79 (5)%	0.40 (10)%	0.6 (2)%
K	3.5 (3)%	4.2 (3)%	1.6 (4)%	2.96%	4.1 (3)%	3.14 (6)%	1.87 (6)%	2.2 (1)%
Ca	1.4 (1)%	1.3 (1)%	3.0 (6)%	1.44%	1.1 (1)%	0.8 (1)%	1.1 (1)%	1.2 (2)%
Ti	200 (100)	170 (40)	100 (40)	66	290 (60)	130 (30)	90 (20)	30 (10)
Cr	20 (10)	23 (8)	16 (6)	9	22 (4)	11 (2)	14 (4)	8 (2)
Mn	100 (10)	70 (10)	69 (8)	43	170 (30)	160 (20)	59 (9)	330 (80)
Fe	0.2 (2)%	0.21 (5)%	0.14 (5)%	780	0.39 (8)%	0.12 (3)%	0.13 (2)%	360 (90)
Ni	ND	ND	ND	ND	ND	ND	ND	ND
Cu	16 (7)	15 (1)	9 (2)	10	20 (3)	18 (1)	9 (1)	10 (1)
Zn	21 (4)	60 (10)	23 (3)	42	50 (6)	90 (30)	42 (4)	49 (6)
As	ND	ND	ND	ND	ND	ND	ND	ND
Br	18 (2)	40 (20)	23 (4)	7	12 (4)	38 (8)	11 (1)	80 (30)
Pb	ND	ND	ND	ND	ND	ND	ND	ND
Rb	18 (7)	26 (1)	16 (4)	18	19 (5)	19 (2)	8 (2)	33 (6)
Sr	36 (8)	160 (60)	100 (20)	130	28 (4)	50 (8)	15 (1)	120 (40)
Mo	ND	ND	ND	ND	ND	ND	ND	ND
I	ND	ND	ND	ND	ND	ND	ND	ND
Ba	40 (20)	50 (20)	24 (6)	41	27 (9)	50 (10)	14 (2)	32 (5)

Element	*Polystichum polyblepharm*		*Boehmeria nivea*		*Glechoma hederacea*		*Taraxacum officinale*	
	South ($n=1$)	North ($n=5$)	South ($n=4$)	North ($n=3$)	South ($n=3$)	North ($n=4$)	South ($n=3$)	North ($n=4$)
P	ND[a)]	900 (200)	0.11 (3)%	0.13 (2)%	0.35 (2)%	0.21 (1)%	ND[a)]	0.31 (5)%
S	0.17%	0.21 (2)%	0.37 (5)%	0.30 (2)%	0.34 (2)%	0.31 (2)%	0.33 (3)%	0.26 (2)%
Cl	0.30%	0.47 (9)%	1.2 (1)%	1.0 (2)%	0.8 (2)%	1.1 (2)%	2.2 (6)%	0.53 (7)%
K	1.20%	2.0 (3)%	1.4 (2)%	1.50 (8)%	4.6 (2)%	3.2 (2)%	5(1)%	7.5 (4)%
Ca	0.69%	0.6 (1)%	3.6 (5)%	3.9 (1)%	2.3 (3)%	2.1 (2)%	2.1 (1)%	1.20 (9)%
Ti	121	50 (10)	230 (40)	110 (20)	50 (10)	24 (2)	330 (70)	58 (7)
Cr	20	4 (1)	16 (5)	16 (2)	4 (1)	3 (1)	50 (10)	11 (2)
Mn	62	37 (5)	140 (20)	80 (10)	82 (5)	100 (20)	130 (20)	100 (30)
Fe	0.19%	500 (200)	0.35 (8)%	0.15 (3)%	500 (100)	290 (20)	0.5 (1)%	500 (100)
Ni	ND	ND	ND	ND	ND	ND	ND	ND
Cu	10	10 (2)	13 (2)	13 (1)	11 (1)	11 (1)	12 (1)	9 (1)
Zn	34	60 (10)	43 (7)	32 (2)	46 (8)	53 (3)	60 (10)	44 (8)
As	ND	ND	ND	ND	ND	ND	ND	ND
Br	2	10 (3)	14 (4)	8 (4)	18 (1)	19 (3)	30 (20)	80 (20)
Pb	ND	ND	ND	ND	ND	ND	ND	ND
Rb	13	10 (2)	13 (3)	14 (7)	10 (2)	11 (2)	19 (3)	50 (10)
Sr	20	60 (20)	90 (20)	118 (1)	51 (6)	110 (10)	64 (5)	120 (10)
Mo	ND	ND	ND	ND	ND	ND	ND	ND
I	ND	ND	ND	ND	ND	ND	ND	ND
Ba	22	90 (20)	46 (8)	40 (7)	89 (8)	45 (5)	50 (20)	80 (10)

a) Overlapping with the peak of Si. ND, not detected; *n*, number of samples.

officinale, the Ca content varied from 1.1% for *A. vulgaris* and *P. cuspidatum* to 3.6% for *B. nivea*, and the Sr content deviated from 28 ppm for *A. vulgaris* to 100 ppm for *P. decursivum*; the same trends were also observed in the case of the northern plants. *B. nivea* and *P. decursivum* contained Ca at higher levels than K,[6)] whereas the others contained K at higher levels than Ca. In this connection, it is also interesting that all plants of Family Compositae tested had a high K content, 3.1—7.5%, and high ratios of K to Ca, 2.3—6.3. In the comparison of metals profiles of the same kind of plant between the northern and southern locations, no significant differences were observed for the content of S, Cl, K, Ca, Cu, *etc.* The overall analytical results suggested that the distinction in metal profiles among different specimens of the same kind of plant growing on different soil types was less than the distinction among the different kinds of plants growing at the same site. On the other hand, the contents of Ti, Fe, Sr, Ba, *etc.* apparently depended on the soil type on which the plant was grown. Ti and Fe were contained in the plants growing in the southern area at higher levels, and Sr and Ba were contained in those at lower levels than in the plants growing in the northern area. This suggests some correlation between the metal profiles of the plants and the soil types.

To elucidate the relationship between the metals profile and inorganic constitution of soil, the soils on which the plants were grown were analyzed by the X-ray fluorescence spectrometry using the internal standard substances.[5)] The analytical results are shown in Table II. From Tables I and II, the element concentrations of the plants or soils for the southern area can be compared with those for the northern area. The elements which were contained in either the

TABLE II. Analytical Results (% or ppm) for Some Soils by X-Ray Fluorescence Spectrometry

Element	*Petasites japonicus*		*Peucedanum decursirium*		*Artemisia vulgaris*		*Polygonum cuspidatum*	
	South ($n=3$)	North ($n=5$)	South ($n=3$)	North ($n=1$)	South ($n=4$)	North ($n=5$)	South ($n=3$)	North ($n=5$)
Si	12.9 (4)%	26 (1)%	14.18 (5)%	14.6%	6.1 (1)%	16.9 (6)%	7.4 (2)%	15 (1)%
K	1.93 (3)%	4.1 (1)%	0.9 (2)%	1.72%	0.74 (1)%	1.87 (4)%	0.99 (3)%	1.69 (3)%
Ca	2.04 (2)%	0.90 (3)%	1.14 (1)%	0.25%	1.49 (2)%	0.22 (1)%	1.51 (3)%	0.54 (8)%
Ti	0.83 (1)%	0.70 (2)%	0.48 (1)%	0.32%	0.31 (2)%	0.31 (1)%	0.32 (1)%	0.26 (2)%
Mn	0.37 (1)%	0.17 (1)%	0.13 (1)%	790	0.13 (1)%	400 (10)	0.12 (1)%	640 (70)
Fe	11.93 (7)%	8.5 (2)%	4.84 (4)%	3.53%	4.3 (2)%	2.72 (5)%	4.7 (6)%	2.88 (8)%
Cu	210 (20)	170 (100)	ND	270	100 (10)	10 (2)	79 (8)	20 (20)
Zn	300 (10)	290 (30)	160 (10)	98	199 (2)	106 (8)	164 (6)	700 (300)
Br	6 (1)	ND	ND	1	3 (2)	ND	ND	ND
Rb	141 (3)	170 (6)	30 (1)	65	40 (2)	60 (1)	40 (2)	60 (2)
Sr	131 (5)	180 (4)	61 (2)	44	63 (9)	33 (9)	71 (5)	59 (9)

Element	*Boehmeria nivea*		*Glechoma hederacea*		*Taraxacum officinale*	
	South ($n=4$)	North ($n=3$)	South ($n=3$)	North ($n=4$)	South ($n=3$)	North ($n=4$)
Si	8.9 (8)%	13.0 (7)%	8 (2)%	41 (5)%	7 (1)%	16 (1)%
K	0.87 (5)%	1.69 (8)%	1.2 (2)%	3.4 (1)%	0.81 (7)%	1.54 (8)%
Ca	1.40 (4)%	0.77 (3)%	2.35 (9)%	0.75 (5)%	1.51 (6)%	0.34 (2)%
Ti	0.30 (2)%	0.21 (1)%	0.6 (1)%	0.45 (4)%	0.32 (1)%	0.31 (1)%
Mn	0.13 (1)%	618 (2)	0.28 (4)%	750 (60)	0.13 (1)%	510 (20)
Fe	5.0 (9)%	2.42 (2)%	9 (2)%	4.7 (1)%	4.4 (1)%	2.7 (1)%
Cu	80 (30)	50 (20)	190 (30)	ND	170 (70)	150 (40)
Zn	140 (10)	120 (2)	402 (8)	180 (20)	200 (20)	108 (6)
Br	ND	ND	15 (2)	ND	ND	2 (1)
Rb	45 (3)	61 (1)	110 (10)	109 (7)	42 (2)	54 (1)
Sr	60 (4)	65 (1)	150 (10)	105 (6)	70 (2)	42 (2)

ND, not detected; n, number of samples. Standard deviation are given in parentheses.

TABLE III. Comparison in Element Concentration of Soil or Plant between South and North

Plant	Plant		Soil	
	South	North	South	North
Petasites japonicus	Mn	K, Zn, Br[a], Sr[a]	Ca, Ti, Mn, Fe	Si, K, Rb, Sr
Peucedanum decursivum	P, S, Ca, Mn, Fe[a], Br	K, Zn, Ba[a], Sr	Ca, Ti, Mn, Fe, Sr	K, Cu, Rb
Artemisia vulgaris	Cl, K, Ca, Ti[a], Cr[a], Fe[a]	Zn, Br[a], Sr[a], Ba	Ca, Mn, Fe, Cu, Sr	Si, K
Polygonum cuspidatum	Ti[a], Cr, Fe[a]	Mn, Br[a], Rb, Sr[a], Ba[a]	Ca, Ti, Mn, Fe, Cu	Si, K, Zn
Boehmeria nivea	S, Ti[a], Fe[a], Zn	Sr[a]	Ca, Ti, Mn, Fe	Si, K, Ca
Glechoma hederacea	P, K, Ti[a], Fe[a], Ba	Sr[a]	Ca, Mn, Fe, Cu, Zn	Si, K
Taraxacum officinale	Cl, Ca, Ti[a], Cr[a], Fe[a], Cu	K, Br[a], Rb, Sr[a], Ba[a]	Ca, Mn, Fe, Sr	Si, K

a) The difference is large.

northern or southern area at significantly higher levels are listed in Table III. As shown in Table III, Si and K were contained in the northern soils at higher levels; on the other hand, Ca, Ti, Mn, Fe, Cu, *etc.* were contained in those at lower levels than in the southern soils. These results are almost compatible with the characteristics of the metal profiles of the corresponding plants. However, only a slight difference in Cu content between the southern and northern plant samples was observed, although Cu existed at apparently higher levels in the southern soils than in the northern soils.

Of special interest is the fact that plants at the northern locations (low Sr-content) contained Sr at higher levels than the southern plants (Sr-rich soils). The typical examples are *A. vulgaris* (50 ppm Sr in the leaves against 33 ppm Sr in the northern soil; 28 ppm Sr against 63 ppm Sr in the southern soil) and *T. officinale* (120 ppm Sr against 42 ppm Sr in the northern soil; 64 ppm Sr against 70 ppm Sr in the southern soil). This unexpected result may be interpreted as follows. In spite of the lower Ca content in the northern soils, the difference in Ca content between the northern and southern plants was little, suggesting that the uptake of Ca, one of the essential elements, is biologically regulated to some extent. In other words, Ca in the northern soils was absorbed into the plant body at higher ratios than the Ca concentration in the soil. Because of the similarity of physicochemical properties between Ca and Sr, the biological uptake of Ca is generally accompanied by the Sr uptake.[6,7] The ratios of Sr to Ca for the northern soils were 2- to 4-fold higher than those for the southern soils, although Sr itself was contained in the northern soils at a lower level. The Sr uptake by northern plants is presumed to take place in conjunction with Ca uptake. Consequently, the plants growing on the low Sr-containing soil came to

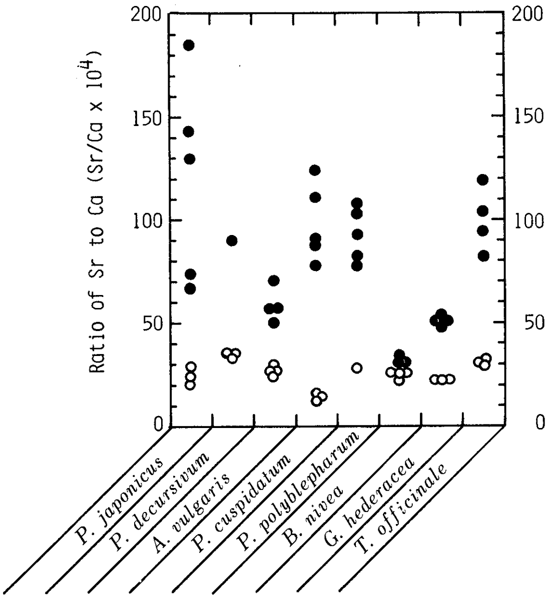

Fig. 2. Ratio of Strontium to Calcium Contents in Plants

●, the plant growing on northern area; ○, the plant growing on southern area.

contain Sr at higher levels than the plants growing on the Sr-rich soils. Figure 2 shows the ratio of Sr to Ca concentration in the plant leaves for several kinds of plants growing on the different soil types. The ratios for southern plant samples lie within the range of 10—40, and those for northern plant samples from 50 to 150. These values of Sr/Ca were found to be closely related to those in the soils, 40—65 and 85—200 for the southern and northern soils, respectively. These results clearly suggest that the Sr content of a plant (or a crude drug) is an important factor for identifying the producing district of a crude drug by its metals profile. The Sr content (or Sr/Ca) of plant leaves reflects the ratio of Sr to Ca of the soil, one of the geological features. Indeed, the authors' X-ray fluorescence spectrometric studies of many crude-drug samples have demonstrated that the Sr content of licorice root depends on the producing district, namely Seihoku- and Tohoku- Kanzo in Japanese.[3] Further, the ratio of Sr to Ca is an essential factor for identifying the exact producing district in the cases of Coptidis Rhizoma and Phellodendri Cortex.[1]

The relationship between a metals profile and the vegetational periods of plants, being important factor as well as the soils on which plants are grown, is under investigation. We also plan to carry out experiments similar to this work with respect to many kinds of monocotyledons in order to confirm valuable information obtained here.

Conclusions

The present analytical results on plant leaves and the soil on which they are grown can be summarized as follows: (1) Even if the plants are growing on the same soil types, the metals profile depends considerably on the kind of plant. (2) The metals profile of the same kind of plant is also influenced by soil types on which the plants are grown. The extent varies widely from element to element. Ca, Cu, S, Cl, and K are less sensitive to the inorganic constitution of soils, whereas Ti, Fe, Sr, and Ba are very sensitive. (3) Sr content of a plant is more closely related to the ratio of Sr to Ca than to the Sr content itself of the soil.

This information is essential in the identification of the producing district (or original plant) of a crude drug by its metals profile.

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References and Notes

1) Part VI: Y. Mino, H. Torii, and N. Ota, *Chem. Pharm. Bull.*, **38**, 1936 (1990).
2) Y. Mino and N. Ota, *Chem. Pharm. Bull.*, **32**, 591 (1984).
3) Y. Mino, Y. Tsukioka, and N. Ota, *Chem. Oharm. Bull.*, **32**, 571 (1984).
4) Y. Mino and N. Ota, *Chem. Pharm. Bull.*, **38**, 709 (1990).
5) K. Matsumoto and K. Fuwa, *Anal. Chem.*, **51**, 2355 (1979).
6) In *P. decursivum*, the Ca content was always more than the K content for any southern plant samples ($n=3$), whereas the opposite result was observed for the northern plant sample ($n=1$).
7) R. D. Goodenough and V. A. Stenger, "Comprehensive Inorganic Chemistry," ed. by A. F. Trotman-Dickenson *et al.*, Pergamon Press, Oxford, 1973, p. 591.
8) E. J. Underwood, "Trace Elements in Human and Animal Nutrition," 4th ed., Academic Press, New York, 1977, p. 445.