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Variation of Mineral Elemental Contents in the Root of *Rheum tanguticum* (Polygonaceae)-A Famous Tibetan Medicine from Different Habitat in Qinghai-Plateau

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The root of *Rheum tanguticum* and its soil, collected from 30 different habitats, were analyzed for 11 mineral elements, soil pH and soil organic material (SOM). Most elements vary over a wide range, depending on geo-environmental factors and different soil characteristics. The mineral element data were evaluated by multivariate methods, *i.e.*, principal component analysis and correlation analysis to reveal the distribution pattern of elements in root and in soil. Effects of environmental factors (including altitude, longitude, latitude and four soil principal components) on the plant minerals were evaluated. Four principal components P_1 (K and Ca factor), P_2 (Cu factor), P_3 (Mg factor), P_4 (Zn and Se factor) of plant elements and four principal components S_1 (Fe factor), S_2 (Mg factor), S_3 (SOM factor), S_4 (Se factor) of soil were selected, which reflected the main pattern of minerals. It can be seen that P_1 had positive correlation with S_3 ($p < 0.05$) and latitude ($p < 0.05$); P_2 showed positive correlation with S_2 ($p < 0.01$), longitude ($p < 0.05$) and latitude ($p < 0.05$), but had negative correlation with altitude ($p < 0.01$); P_3, P_4 had no clear significant correlations with the environmental factors determined in this experiment. There are many inter-correlations between mineral elements in plant and inter-correlation between elements, pH and SOM in soil. Thus, it is important to consider the effects of different environmental factors and inter-correlation on the elemental distribution.

Key Words: *Rheum tanguticum*, Mineral elements, PCA, Tibetan medicine, Environmental factor.

INTRODUCTION

Rhubarb is the most analyzed plant and known for its numerous medicinal use in Chinese medicine and modem literature. *Rheum tanguticum* Maxim. Ex Balf (Polygonaceae) is a famous perennial herbal medicine distributed in China, especially in Qinghai-Tibet Plateau, which grows in alpine meadows, forest edges, furrow valleys under forests, shrubs, the edges of the road, at altitudes from 2300-4700 m[1]. Qinghai is the main producing area of *Rh. tanguticum*, the yield in Guo Luo state has the vast majority consisting of about 63 % total wild production in Qinghai. There are rich resources, owing to its special habitat, short duration of frost-free period, high altitude, growing slowly, abundance time of physical accumulation, hence, this region produces a high quality rhubarb. It is well known as "Xining Da Huang"[2]. The rhurarb not only commonly used in China but also are widely consumed all over the world. The fleshy root and rhizome are usually used as medicine, with the effect of purgation, purging heat, loosening the bowels, curing gastric and renal disorders, removing bacterial dysentery, removing heat from the blood, clearing toxins away, promoting blood circulation, removing blood stasis[3]. Along with these important medical functions, combing other various pharmacological properties, it is called the name "JiangJun". Most studies on rhubarb reported in the literature pertain to the chemical constituents and the efficacy, as well as the variety identification and quality evaluation and so on[3-8]. To our best of knowledge, there are some literatures about determination of a few inorganic elements in this herb[9-11]. However, there are insufficient data available on the characteristics or quantitative analysis of mineral elements in *Rh. tanguticum* under different wild environmental conditions from the Qinghai-Plateau.

It is supposed that inorganic elements may be coordinated with organic compounds present in the herb and can be used to treat for different disease. However, ecological environment is one of the crucial factors influencing the formation and the variation of chemical compositions and elements concentrations. Elemental uptake by a plant is its characteristic property and may depend on soil type, pH, fertilizer and organic content, different climatological/geo-environmental factor, species, *etc.*[12].

Thus, the main objective of the present study is to determine quantitatively some mineral elements in *Rh. tanguticum* root and the soil itself from different natural habitats. The eleven mineral elements in 30 plant samples and its soil samples were measured. The effect (if any) of environmental factors (including altitude, longitude, latitude and soil characteristic) on the mineral pattern in root of *Rh. tanguticum* were investigated. The principal component analysis was applied to explain the pattern of mineral content and the association (if any) between minerals in *Rh. tanguticum* roots and environmental factors. With these results we wish to provide a basis for further experiments.

EXPERIMENTAL

For the analysis of nine elements (K, Ca, Mg, Fe, Cu, Mn, Zn, Ni and Cr), an atomic absorption spectrophotometry (TAS-986, PuXi Tongyong Company, Peking, China)with air oxyacetylene flame was used. Selenium was determined with hydride-generation technique and phosphorus was measured by a colorimetric method with spectrophotometer(721, No. 3 Analysis Apparatus Company of Shanghai) and the absorbance at 400 nm. pH was determined with pH electrode.

Working standard solutions were prepared from standards of single minerals. The concentrations of these working standard solutions (five standards for each element)were in the linear range of each element in the plant and soil samples. The recovery percentages of each element were 98.26-103.85 %. Therefore, the variation coefficients were all below 5 %. Nitric acid (HNO_3), perchloric acid ($HClO_4$) and hydrogen peroxide (H_2O_2), vanadate-molybdate were purchased from the Chemical Reagents Company of Shandong in China and were reagent analytical grade quality. All solutions and dilutions were prepared with double-distilled deionized water. The data were given as the mean of five parallel samples, each parallel sample was determined three times.

Sample preparation: *Rh. tanguticum* roots were collected in August, 2007 from the Qinghai plateau at altitude from 2200-4500 m in 30 different localities and its soil samples from these 30 localities were collected. For each site, at least 5 individuals were picked randomly and 5 corresponding soil samples at least 100 cm deep from different positions separated by more than 10 m, were taken and then mixed together. The roots were slightly washed in field and again in the laboratory with bidistilled water in order to remove soil and avoid any surface contamination. These were then dried at 60 ºC to constant weight and crushed to a homogeneous fine powder (100 mesh) in agate mortar. Accurately weighed samples of root (*ca.* 4 g each) and 10 mL of HNO_3 were mixed and then 2 mL of H_2O_2 were added after 1 h and the samples were kept at 25 ºC for 12 h before heating on a hot-plate at 70-100 ºC for 3 h. Samples were allowed to cool and then transferred in 100 mL volumetric flasks for the analysis.

Soil samples (0.5 g each) were first sieved from 100 sieve pore and then decomposed in 15 mL of HNO_3 and heated, 2 mL of $HClO_4$ were added into each sample and hold boiling for 6 h and then transferred to 100 mL volumetric flasks for the analysis.

Environmental factors: The locations and geographical factors relevant to each sample, including altitude, longitude and latitude, were determined with a GPS apparatus (Unistrong Science & Technology Co, Ltd. Peking, China) (Table-1).

Data analysis: The data were analyzed with the SPSS 13.0 software package. The principle component analysis and correlation analysis were used for the analysis of the plant and soil mineral distribution patterns in different environments and to further study their relationship, as well as the inherent relation.

TABLE-1
ENVIRONMENT FACTORS IN 30 SAMPLES

Sample	Sites	Altitude (m)	Latitude	Longitude
1	Guomaying, Guinan	2770	35º53.61'	100º57.27'
2	Qunjia, Huangzhong	2882	36º17.5'	101º40.71'
3	Maixiu^2, Zeku	2891	35º18.61'	101º56.32'
4	Maixiu^1, Zeku	2944	35º16.30'	101º55.98'
5	Yeniugou, Qilian	3228	38º17.38'	99º49.57'
6	Tongde county	3294	35º15.43'	100º39.49'
7	Duofudong, Zeku	3309	35º14.96'	101º52.26'
8	Zhamashi, Qilian	3412	38º21.05'	99º49.48'
9	Makehe^2, Banma	3415	32º46.45	100º47.07'
10	Duogongma, Banma	3417	33º6.78'	100º34.13'
11	Lajia, Maqin	3433	34º34.31'	100º33.48'
12	Jungong, Maqin	3450	34º38.94'	100º36.49'
13	Banma county	3517	32º55.44'	100º45.92'
14	Heri, Zeku	3517	35º13.9'	101º0.13'
15	Qiakeri, Zeku	3690	35º2.97'	101º29.09'
16	Jilong, Zeku	3710	35º9.56'	101º12.2'
17	Zhiqin^2, Banma	3774	32º39.82'	100º30.94'
18	Zhiqin^1, Banma	3785	32º42.26'	100º26.60'
19	Duogongma ,Banma	3832	33º05.48'	100º29.08'
20	Jika^3, Banma	3891	32º51.07'	100º12.82'
21	Jika^2, Banma	3933	32º54.70'	100º10.12'
22	Daka, Banma	3941	32º55.91'	100º09.37'
23	Shanggongma,Gande	4032	33º52.13'	99º39.75'
24	Deang, Dari	4041	32º17.39'	100º25.7'
25	Jianshe, Dari	4055	33º40.42'	99º27.16'
26	Wosai, Dari	4090	33º33.49'	99º56.61'
27	Dawu county	4092	34º28.61'	100º23.06'
28	Makehe^1, Banma	4158	32º58.74'	100º20.34'
29	Manzhang, Dari	4358	33º17.55'	100º22.91'
30	Jika^1, Banma	4484	32º55.57'	100º18.67'

RESULTS AND DISCUSSION

Element distribution patterns in *Rh. tanguticum* roots: The biological effects of estimated elements (K, Ca, Mg, Fe, Cu, Mn, Zn, Ni and Cr) in living systems strongly depend on their concentration[13,14] and thus should be carefully controlled[15], especially when herbs and their products are used in human medicine as in the case with *Rh. tanguticum*. The concentrations of 11 minerals in *Rh. tanguticum* roots are given in Table-2. The relative standard deviations were in the range from 0.2-2.5 % confirming good reproducibility of the applied method. In 30 samples, it was observed that the levels of the same element showed a wide variability in different locations, which indicated that the ecological environment and the types of soil were important factors affecting the content of mineral elements in this plant.

The mean concentration values of 11 elements decreased as follow: P > Ca > Mg > K > Fe > Mn > Cr > Zn > Ni > Cu > Se. Selenium, Cu and Ni were found in trace (< 10 μg g^{-1}), while the concentrations of the other elements were much higher, especially P, Ca, K, Mg (> 1800 μg g^{-1}) and Fe (> 100 μg g^{-1}), which play an important role in the metabolism for living organisms[16].

TABLE-2
MEAN CONCENTRATION AND STANDARD DEVIATION (μg/g) OF ELEMENTS IN *Rh. tanguticum* ROOTS

Sample	k	Ca	Mg	P	Fe	Cu	Mn	Zn	Ni	Cr	Se
1	2025.4	1963.8	2086.2	942.1	76.8	2.2	21.3	33.0	5.5	42.4	1.37
2	1899	2101.8	1526	4553.9	131.4	2.9	27.8	33.0	5.7	42.7	1.16
3	2012.4	1932.3	2077	2600	225.4	4.1	30.9	30.8	6.3	42.9	1.44
4	1999.5	801.3	1390.3	5323.7	100.1	3.1	19.8	30.9	4.6	42.4	1.31
5	1759.3	2455.9	2084.7	1534.2	57	2.5	148.2	30.7	8.1	42.8	1.00
6	1906.4	2077	2254.5	3192.1	137.1	4.9	31.2	30.7	4.1	42.6	1.13
7	1746.7	2548.8	1740.2	912.5	66.9	2.0	116.2	31.8	10.5	43.4	1.61
8	1832.7	2507.9	1833.3	1327	111.4	4.8	136.4	30.7	13.3	43.5	1.25
9	1852.8	2400.1	1778.2	2244.7	109.6	4.0	67.1	30.9	6.2	42.4	1.22
10	2019.7	2243.9	1998.7	3132.9	90.9	2.3	87.4	31.0	4.8	42.4	0.83
11	1935.8	2355.7	1783.6	1948.7	111.6	5.1	32.5	31.2	8.2	42.7	1.31
12	1936.5	2047.6	2216.2	2422.4	196.5	3.1	31.6	31.3	3.6	42.8	1.20
13	1854.9	2266.6	2125.3	3606.6	131.6	3.2	40.5	30.9	5.2	42.1	1.27
14	1882	2116.4	1993.5	1711.8	64.8	2.2	19.1	31.4	3.6	42.4	1.31
15	1664.8	2426	1759.4	1208.6	56.9	2.4	99.0	31.3	7.5	42.9	1.19
16	1926.9	1805.7	1892.4	4909.2	85.7	3.3	19.6	30.7	5.5	42.4	1.31
17	1926.2	2148.6	1879.4	2540.8	70	3.0	115.1	31.3	4.7	41.9	1.21
18	1886.1	2163.4	1897.3	1948.7	81.4	1.8	47.4	31.1	3.9	42.0	1.15
19	2153.3	699.5	1560.2	4850	103.1	4.1	33.8	32.6	6.2	41.5	1.19
20	2035.2	1903.9	1928.7	3192.1	115.5	2.4	23.9	30.9	4.1	41.8	0.56
21	1866.7	2276	2172.4	5501.3	109.6	1.6	131.7	31.7	7.2	41.7	1.39
22	1867.1	1790.6	1770.8	2718.4	132.2	3.0	24.9	30.8	4.1	41.7	0.62
23	1874.9	2239.2	1845.1	1504.6	88.6	2.2	45.6	31	4.6	42.4	1.19
24	1901.5	2364.4	1894.3	3725	240.3	2.3	60.6	31.2	8.5	42.6	1.06
25	1831.8	2341	1783.6	2363.2	103.4	2.5	47.8	31.6	5.9	42.6	1.12
26	1854.1	2165.5	2323.4	2244.7	145.5	2.9	55.2	30.8	5.2	42.3	1.24
27	1876.4	2147.9	1850.6	3192.1	72.3	2.5	22.6	30.7	4.1	42.4	1.19
28	1963.5	2204	2116.5	2836.8	91.5	2.7	51.1	31.3	6.0	41.7	1.23
29	1951.1	2170.8	2152.1	2007.9	179.9	2.8	89.4	31.4	4.6	42.3	1.09
30	1782	2404.1	1938.3	971.7	128.1	2.0	58	30.7	5.9	41.8	1.58
Mean	1900.8	2102.3	1921.7	2705.6	113.8	2.9	57.8	31.2	5.9	42.4	1.19
SD	97.9	416.2	217.9	1311.4	46.4	0.9	39.1	0.6	2.1	0.5	0.224

To study mineral element patterns in the herb from different habitat, a chemometric approach was also used. In the first step of the statistic evaluation, the principal component analysis was used to reduce the dimensionality of the original data matrix retaining the maximum amount of variability. It provides a new set of variables (principal components, PCs) which facilitates the discovery of patterns hidden in the data set[17,18]. Therefore, the method was used to analyze the mass mineral element data in plant roots. It removes the highly inter-correlated nature of variations in mineral element concentrations. The eigenvalues and the percentages of variance (%) of principal components in plant elements were presented in Table-3. The first four principal components represent 70.745 % of all the variables information in plant root mineral content. One of the main purposes of principal component analysis is to identify variables that are substantially meaningful. According to the Kaiser criterion[19], only the first four PCs were retained because subsequent eigenvalues are all less than one, which can basically represent and reflect the main information and the mutual relationship of the controlled characteristics among the mass indexes of plant root elements.

Another rotated component matrix of the first four principal components was given in Table-4. In the first principal component, Ca and K had large loading variable, which are multi-functional nutrients that form an essential part of many important enzymes[20]. Thus, the first principal component can be considered as P_1. In the second component, Cu had the largest loading variable, so it is P_2. Cu is essential for a variety of biochemical processes and is needed for certain critical enzymes to function in the body. It is also involved in the functioning of the nervous system, in maintaining the balance of other useful metals in the body such as zinc and molybdenum and is necessary for the normal function of the immune system[21]. It can be seen that magnesium had the largest loading matrix in the third component. Magnesium is not only an important electrolyte but is also responsible for proper nerve and muscle function. It can works as co-factor in more than 300 metabolic reactions[22] as well as a constitutive element of chlorophyll

TABLE-3
EIGENVALUES AND PERCENTAGE OF VARIANCE (%) PRINCIPAL OF PLANT ROOT ELEMENTS

Component	Initial Eigenvalues		
	Total	Variance ratio (%)	Cumulative ratio (%)
1	3.434	31.221	31.221
2	1.724	15.677	46.898
3	1.569	14.268	61.165
4	1.054	9.579	70.745
5	0.870	7.911	78.655
6	0.727	6.611	85.267
7	0.630	5.728	90.995
8	0.351	3.187	94.182
9	0.317	2.883	97.065
10	0.193	1.750	98.816
11	0.130	1.184	100.000

TABLE-4
LOADING MATRIX OF THE FIRST FOUR COMPONENTS IN THE ROTATED COMPONENT MATRIX OF PLANT ROOT ELEMENTS

Element	Principal component			
	1	2	3	4
K	-0.850	0.009	0.116	0.072
Ca	0.864	-0.021	0.281	-0.022
Mg	0.262	-0.225	0.825	-0.080
P	-0.690	0.046	-0.172	-0.101
Fe	-0.281	0.375	0.691	-0.039
Cu	-0.284	0.800	0.107	-0.127
Mn	0.710	0.121	-0.205	-0.020
Zn	-0.272	-0.176	-0.229	0.759
Ni	0.517	0.639	-0.266	0.233
Cr	0.513	0.633	-0.018	0.174
Se	0.217	0.188	0.079	0.751

which is involved in enzyme reactions, it is P_3. The last factor may be deduced by analogy it is P_4. Zinc is the component of more than 270 enzymes[23] and its deficiency in the organism is accompanied by multisystem dysfunction. Additionally, Zn is responsible for stimulating growth of epidermal and epithelial cells[24], sperm manufacture, fetus development and proper function of immune response[25]. In this study, K and Ca, Cu, Mg, Zn and Se were the four main component elements, can represent the majority of the mineral pattern in *Rh. tanguticum* roots. These elements play an important role in its pharmacology, for example, the effect of loosening the bowels, purgation and constipation may be attributed to considerable amounts of K present in them[26].

The correlation test was performed to investigate relationship among the mineral contents in examined *Rh. tanguticum* roots. The entire data were subjected to a statistical analysis and correlation matrices were produced to examine the inter-relationship between the investigated mineral concentrations. The values of correlation coefficient between mineral element concentrations are shown in Table-5. The correlations between K-P, Ca-Mn, Mn-Ni, Cr-Ni were positive correlation ($p < 0.01$); K-Ca, K-Mn, Ca-P were negative correlation ($p < 0.01$); Ca-Mg, Ca-Ni, Ca-Cr were positive correlation ($p < 0.05$); K-Ni, K-Cr, P-Cr were negative correlation ($p < 0.05$). K, Ca, Mn, P four element content in rhubarb correlated with each other and can be combined together to be considered as a whole of the P_1 principal component, in agreement with the PCA result. Copper, Cr, Ni combined together formed P_2 principal component. Magnesium and Fe were both vital to photosynthesis, they correlated and formed P_3 principal component. Though Zn and Se had no clear significant relationship, they combined and composed P_4 principal component. Four principal components represented the rhubarb elements distribution pattern.

Distribution pattern of mineral in the soil samples: Soil characteristic together with environmental conditions play an important role in the nutrients contents[27]. Therefore, concentrations of the corresponding 11 mineral elements in the 30 soil samples were determined (Table-6). The mean concentration values of 11 elements were ranked as follow: Ca > Mg > Fe > K > P > Mn > Cr > Zn > Ni > Cu > Se. Thus, comparing to the plant it showed a large different distribution. The fact may be attributed to factors such as preferential absorbability of a plant species for a specific element[28] and elemental composition of the soil where the plants were existed.

In order to analyze the inherent relationship between soil minerals, the principal component analysis was used. According to the loading matrix, there were four principal components, which account for 73.768 %. The data were given in Table-7. In terms of the same criterion, a rotated loading matrix of the first four components was given in Table-8. The four soil principal components were different from those in the plants. In the first component, Fe had the largest loading matrix and we considered it as S1. In the second component, Mg had the large loading matrix and could be considered as S_2. In the third component, SOM had the large loading matrix, so we called it as S_3. In the fourth component, Se had large loading matrix and we called it as S_4. In soil, the four principal components Fe, Mg, SOM and Se can generally reflect the habitat in which the plant had existed.

The values of correlation coefficient between mineral element concentrations, pH, SOM are shown in Table-9. The correlations between K-Mn, Ca-Mg, pH-Ca, pH-Mg, P-SOM, Fe-Mn, Fe-Ni, Cu-Fe, Cu-Mn were positive correlation ($p < 0.01$); Fe-Zn, Cu-Zn, Mn-Zn, Ni-pH were negative correlation ($p < 0.01$); Cr-Mg, Cu-Ni, Mn-Ni were positive correlation ($p < 0.05$); P-Zn, P-pH were negative correlation($p < 0.05$). S_1 mainly consisted of Fe, Cu, Mn, Zn, Ni elements and they formed an integrated unit. If any of their contents changed, other four elements content changed correspondingly, their variations had the same trend. Mg, Ca, pH correlated with each other and they formed the S_2 principal component. P and SOM positively correlated and formed the S_3 principal component. Se had no clear significant relationship with other

TABLE-5
CORRELATION MATRIX OF ELEMENTS DATA IN *Rh. tanguticum* ROOTS (PEARSON CORRELATION)

	K	Ca	Mg	P	Fe	Cu	Mn	Zn	Ni	Cr
Ca	-0.694**	–	–	–	–	–	–	–	–	–
Mg	-0.050	0.414*	–	–	–	–	–	–	–	–
P	0.470**	-0.594**	-0.232	–	–	–	–	–	–	–
Fe	0.249	-0.032	0.279	0.185	–	–	–	–	–	–
Cu	0.259	-0.205	-0.084	0.100	0.258	–	–	–	–	–
Mn	-0.485**	0.498**	0.140	-0.262	-0.216	-0.158	–	–	–	–
Zn	0.304	-0.222	-0.252	0.111	-0.109	-0.154	-0.107	–	–	–
Ni	-0.363*	0.365*	-0.184	-0.225	-0.026	0.234	0.623**	0.029	–	–
Cr	-0.426*	0.409*	-0.066	-0.381*	0.058	0.251	0.294	-0.080	0.589**	–
Se	-0.218	0.107	0.011	-0.142	-0.064	0.035	0.099	0.177	0.314	0.265

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

TABLE-6
MEAN CONCENTRATION AND STANDARD DEVIATION (μg/g) OF ELEMENTS, pH AND SOM IN SOIL SAMPLES

Sample	k	Ca	Mg	P	Fe	Cu	Mn	Zn	Ni	Cr	Se	pH	SOM
1	2949.8	12471.8	8668.8	913.7	4952	9.2	565.6	255	38.8	345.4	9.41	8.13	31
2	4393.8	15165.2	13566.6	1378.9	5536.8	19.4	1042.2	247.2	68.4	368.4	9.41	8.09	45.9
3	4460	11196	10334.8	915.3	5576	28.2	966	246.6	54	360.4	9.24	8.14	32.1
4	4139.8	15255	10189.8	1852.6	5335.4	20.8	837.6	245.6	49.4	358.8	5.25	8.11	59.6
5	3560.4	6990.4	9950.8	1605.8	5537	27.2	795	250	64.6	362	8.32	7.97	141
6	4855.6	16305.8	11319.6	1370..5	5296.8	14.8	762	254	38	359	7.92	7.9	31.5
7	4798.4	10626.4	8105.4	2563.1	5480.8	20.2	890.8	250.6	53	365.6	8.95	7.96	141.6
8	3632	3652.8	9132.8	2326.3	5468.6	28	880	253	68	361.2	8.87	7.52	163.9
9	3980.2	13391.8	8940	900.3	5490.6	14.4	832.2	256	98.2	357.4	10.52	7.69	12.3
10	5105	5156	7118	2094.5	5575	15.8	879.6	250.4	89.4	360.2	11.6	7.75	60.2
11	3664.8	14449	11123.6	1620.6	5839.8	46.2	826.6	245.6	119.4	359.8	9.15	7.53	28.7
12	3944.2	14926.6	9417.2	1615.8	5513.2	21.8	742.2	252.8	99.4	355.4	8.16	7.6	61.5
13	6242.2	4629.6	6864.2	1855.6	5593.8	20.8	1017.2	248.2	92	359	9.72	6.63	57.9
14	3984.6	14701.8	9010.4	1612.8	5379.4	17.2	832	252.2	49.4	357.4	7.54	7.97	50.4
15	3762	1509.8	6257	1142.1	5713.2	17.2	814.4	251.2	41.2	356.2	6.4	7.9	77.4
16	4881	10946.6	8918.6	2089.5	5370.8	19.8	834.2	249.4	47.4	358.4	7.45	8.1	40.9
17	5530.8	4636	7659.8	1846.6	5820.8	33.2	1726	245.6	105.4	358.2	9.99	6.55	82.5
18	4500.8	5468.6	6420.6	3279.7	5553.4	15.8	952	251	89	359.4	12.42	5.99	85.4
19	4621.4	6298.8	7395.6	2800	5482.6	15	870.8	246	92.2	352.6	7.28	7.13	60.2
20	3796.4	3163.2	6355.4	902.3	5466.2	13	838	253.6	86.8	357.2	9.2	7.28	20.6
21	5234.8	9011.2	6034.8	3270.7	5777.6	39.2	1706.6	245.8	103.8	356.8	10.34	7.29	115.4
22	3740	3274	5139.4	1497.4	5542.2	15.6	932	248.6	79.2	355.6	6.71	6.57	53.4
23	4735.4	12073	7185.4	900.5	5350.2	15.4	731.8	251.8	89.4	351.8	7.55	7.12	66.9
24	5089.2	12233	7976.4	915.3	5513.8	19.2	980.8	251	93.2	356	8.99	7.86	16.9
25	4755.4	10352.4	7252.4	668.4	5439.2	17.4	786	249.8	93.8	357.2	11.36	7.11	29
26	5113	3307.8	5391.8	895.3	5439	17.8	832.8	250	87.2	354	6.81	6.88	25.6
27	3505.6	13666	7221.6	905.3	5509.8	18.8	932.4	240.6	100.4	355.8	5.3	7.21	23.7
28	5956.4	4328.8	7097.8	2326.3	5584.8	19.8	956.8	247.8	93.4	359.2	8.35	6.65	85.9
29	6650.2	3693.8	7078.8	1378.9	5627.4	25.8	1239.4	246.4	97.4	356.8	5.79	6.75	35.2
30	3593.4	5127.6	7322	3273.7	5433.8	12.6	821.4	246.6	83	357.2	7.36	6.16	129
Mean	4505.9	8933.6	8148.3	1690.8	5506.7	20.7	927.5	249.4	78.8	357.7	8.51	7.4	62.2
SD	873.3	4695.7	1905.0	773.4	169.0	8.0	244.4	3.3	22.9	4.0	1.79	0.6	40.6

TABLE-7
EIGENVALUES AND PERCENTAGE OF VARIANCE (%) PRINCIPAL OF SOIL ELEMENTS

Component	Initial Eigenvalues		
	Total	Variance ratio (%)	Cumulative (%)
1	4.179	32.148	32.148
2	2.421	18.620	50.768
3	1.710	13.154	63.922
4	1.280	9.846	73.768
5	0.981	7.548	81.316
6	0.713	5.487	86.803
7	0.563	4.332	91.136
8	0.298	2.289	93.425
9	0.276	2.123	95.548
10	0.223	1.713	97.261
11	0.187	1.441	98.701
12	0.112	0.860	99.561
13	0.057	0.439	100.000

TABLE-8
LOADING MATRIX OF THE FIRST FOUR COMPONENTS IN THE ROTATED COMPONENT MATRIX OF SOIL ELEMENTS

Element	Principal Component			
	1	2	3	4
K	0.563	-0.250	-0.011	0.099
Ca	-0.107	0.771	-0.323	-0.006
Mg	-0.025	0.921	0.019	0.041
P	0.194	-0.222	0.803	0.032
Fe	0.860	-0.104	0.155	0.072
Cu	0.783	0.287	0.140	-0.053
Mn	0.799	-0.155	0.190	0.048
Zn	-0.674	0.010	-0.308	0.510
Ni	0.658	-0.406	-0.253	0.249
Cr	0.295	0.486	0.498	0.253
Se	0.130	0.006	0.111	0.937
pH	-0.296	0.816	-0.108	-0.072
SOM	0.019	-0.078	0.925	-0.011

elements, it solely formed the S_4 principal component. Four principal components represented the sampling site soil elements distribution pattern.

Effects of environmental factors on plant minerals: Correlation between the distribution pattern of plant minerals and the environmental factors was examined in an attempt to obtain some useful information to guide the cultivation of this medicinal plant under different environmental conditions and to sustain this precious folk plant resource. The root is the important part which contacts with the soil and a variety of elements are transported and absorbed to meet the physiological and physical needs of the plant. Thus, the four major soil components were included in the environment factors.

The correlation coefficient between the four principal components of plant minerals and the environmental factors (including the four soil principal components) are presented in Table-10. P_1 had a positive correlation ($p < 0.05$) with the latitude and S_3, which meant the levels of Ca and K in this herb were affected by the latitude and SOM of sampling. P_2 had a significant positive correlation with S_2 ($p < 0.01$), meanwhile, having a positive correlation with latitude ($p < 0.05$) and longitude ($p < 0.05$). While, P_2 had a negative correlation

TABLE-9
CORRELATION MATRIX OF ELEMENTS DATA IN SOIL SAMPLES (PEARSON CORRELATION)

	K	Ca	Mg	P	Fe	Cu	Mn	Zn	Ni	Cr	Se	pH
Ca	-0.267	–	–	–	–	–	–	–	–	–	–	–
Mg	-0.246	0.704**	–	–	–	–	–	–	–	–	–	–
P	0.148	-0.267	-0.146	–	–	–	–	–	–	–	–	–
Fe	0.347	-0.339	-0.145	0.242	–	–	–	–	–	–	–	–
Cu	0.172	0.052	0.229	0.188	0.703**	–	–	–	–	–	–	–
Mn	0.530**	-0.249	-0.200	0.331	0.661**	0.572**	–	–	–	–	–	–
Zn	-0.348	0.121	0.027	-0.415*	-0.519**	-0.492**	-0.516**	–	–	–	–	–
Ni	0.306	-0.186	-0.330	0.100	0.579**	0.373*	0.414*	-0.263	–	–	–	–
Cr	0.249	0.089	0.437*	0.205	0.330	0.211	0.208	-0.249	-0.098	–	–	–
Se	0.115	-0.032	0.028	0.184	0.165	0.106	0.183	0.271	0.254	0.230	–	–
pH	-0.332	0.588**	0.631**	-0.372*	-0.308	0.035	-0.323	0.250	-0.598**	0.195	-0.091	–
SOM	-0.070	-0.350	-0.073	0.687**	0.178	0.230	0.197	-0.229	-0.115	0.359	0.073	-0.154

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

TABLE-10
CORRELATIONS COEFFICIENTS BETWEEN THE FOUR PRINCIPAL COMPONENTS OF PLANT ELEMENTS AND THE ENVIRONMENTAL FACTORS

	S1	S2	S3	S4	ALT mean	Longitude	Latitude
P1	-0.019	0.093	0.410*	0.184	-0.019	-0.121	0.400*
P2	0.014	0.554**	0.218	-0.158	-0.483**	0.454*	0.457*
P3	0.121	0.215	-0.310	0.038	-0.071	-0.048	0.074
P4	-0.023	0.099	-0.092	0.020	-0.087	0.227	-0.043

($p < 0.05$) with altitude, implying that there were comparatively high contents of Cu in areas at lower altitude. In the four principal components of the plant minerals, it was found that P_2 and P_1 were affected greatly by the environment, indicating that P_2 and P_1 were more sensitive with the external environment change. The plant may regulate the physiological activity to adapt itself to the plateau soil and climate condition by adjusting P_2 and P_1. However, P_3 and P_4 were more immune to disturbance comparing with P_1 and P_2 and were less affected by the environment conditions. From Table-10, it is also found that the S_1 and S_4 had no clear effect on the four principal factors in the plant minerals.

Conclusion

We used the principal component method to analyze the data, but the four principal components can explain about 71 % of the total variation, which implied that other factor influence the mineral element of *Rh. tanguticum* roots and further studies are needed. The variations in the mineral composition of same plants in different samples can be attributed to several factors like the climatic conditions of the region, mineral composition of the soil in which the plant grows, the preferential uptake of a particular plant for certain elements, the species and age of the plant and the SOM. Moreover, these results provided a reference to prescribe the dosage of *Rh. tanguticum* in medicine.

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