

Accumulation dynamics of elements in *Panax notoginseng* during its whole growing seasons

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Abstract

Panax notoginseng is a valuable traditional medicine in China, which could stanch the blood, disperse gore and reduce the pain caused by blood diseases. Understanding the accumulation dynamics of elements during the growth of *PN* is conducive to guiding rational fertilization in production. In this study, 17 elements (B, Na, Mg, Al, P, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, and Pb) in *PN* from different periods were determined by ICP-OES and ICP-MS. The result showed that the change of element accumulation in different periods was consistent with the growth rule of *PN*. Element content in leaves and roots differed during the same period. Therefore, according to the changes of element content in different periods, scientific fertilization can be carried out to improve the quality and yield of *PN*. Besides, to study the accumulation characteristics of *Panax* in the same place, 17 elements in *P. vietnamensis*, *P. stipuleanatus* and *P. bipinnatifidus* were also determined by ICP-OES and ICP-MS. The result showed that *P. vietnamensis* and *P. stipuleanatus* exhibited more similar in elements than the *P. notoginseng* and *P. bipinnatifidus*. HCA and PCA analysis found that different *Panax* species can be distinguished based on element content.

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Keywords: *Panax notoginseng* (*PN*); accumulation of elements; ICP-OES; ICP-MS; PCA

1. Introduction

Panax notoginseng (Burk.) F.H.Chen (Araliaceae), a valuable traditional medicine called Sanqi or Tianqi in Ginseng family has been cultivated for more than 400 years in the southwest of China (Ng, 2010). The

growth cycle of *Panax notoginseng* (*PN*) is slow, and it will be harvested in the third year generally. The roots of *PN* are often used as medicines, such as Yun-Nan-Bai-Yao and Fufang-Danshen-Diwan in China. It has the effects of removing blood stasis, stopping bleeding, reducing swelling and pain (Xia et al., 2017). Since the main active constituents in *PN* are dammarane-type saponins, among which ginsenosides Rg₁, Re, Rb₁, Rd and notoginsenoside R₁ are the five major components (Lau et al., 2003; Wang et al., 2006, 2008, 2012), many studies have focused on the saponin component in *PN*, and this study focuses on the accumulation dynamics of elements during the growth of *PN*.

Modern medical research indicates that the pharmacological activity of Chinese herbal medicine is manifested by the synergistic effect of various components in the body, and inorganic elements play an important role in the metabolism, growth, and development of human body, the occurrence, and development of diseases (Zhao et al., 2013). One of the important ways to clarify traditional pharmacology, toxicology and drug classification is to study the inorganic elements in Chinese herbal medicine (Yang et al., 2019). The metal content of plants has a great impact on the quality (Edzard, 2002), and the content of heavy metals often becomes a bottleneck in the export of medicinal plants, determination, and monitoring of the element content of *PN* is of special significance. Understanding the accumulation dynamics of elements in *PN* is conducive to guiding rational fertilization in production. Some studies on elements of *P. notoginseng* had been done, including the determination of heavy metals and trace elements in *PN*, and the determination of heavy metal content in the soil of *PN* from different producing areas (Xu, 2012; He et al., 2011; Xie et al., 2017; Zhang et al., 2017; Zhao et al., 2014; Lv et al., 2019). However, the accumulation of elements in different growth stages of *PN* was still blank.

At present, the methods for the determination of inorganic elements mainly include atomic absorption spectrophotometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES) (Luo et al., 2015). ICP-MS and ICP-OES provide fast analysis, broad dynamic linear range and simultaneous multielement detection (Nuchdang et al., 2019). To reveal the accumulation of inorganic elements in the different growth period of *P. notoginseng*, 17 elements in *PN* from different periods were determined by ICP-MS and ICP-OES, including B, Na, Mg, Al, P, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, and Pb. The differences of the inorganic elements in the roots and leaves of *PN* were compared, as well as the differences and genetic relationships of the elements among the 4 *Panax* species. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were performed on the results of 27 samples to analyze the main inorganic elements in *PN*. According to the accumulation of elements in the whole growth seasons of *P. notoginseng*, the amount of large and trace elements used in the growth of *PN* can be judged to guide actual production, and it can provide a certain basis for reasonable fertilization to promote the production of high-quality *P. notoginseng*.

2. Materials and Methods

2.1 Sample

24 samples of *P. notoginseng* from different periods and 4 *Panax* species were chosen for the present study (Table 1). *PN* samples were collected from seedbed and field. *PN* seeds have a dormant period of 1 to 2 months, and germination begins at the end of March or the beginning of April. Seedlings are raised in seedbeds from April to December and then transplanted to the field for 2 years of cultivation until harvest. In June and July of the second and third years, flower buds will be removed. Generally, the *PN* root will be harvested at the end of October of the third year.

At the end of March of the first year, we began to germinate the seeds of *P. notoginseng*, and then from April to December, the seedlings of *PN* were cultivated on the seedbed, and then transplanted them into the field. From June of the first year, every month on the 20th we collected the leaves and roots of *PN* until *PN* harvest. The 4 *Panax* species were collected in the same growing area. The sample was washed thoroughly with tap water, followed by ultrapure water, dried at 45 °C for several hours until constant weight, and then pulverized and filtered through a 74 μm (200 mesh) sieve.

2.2 Digestion procedure

The *P. notoginseng* samples were treated according to the following procedures: about 0.300 g of powdered *PN* samples were accurately weighed into a Teflon digestion vessel. 5 mL of concentrated HNO_3 and 1 mL of concentrated H_2O_2 were added to the vessel. Then the vessels were capped, placed in the microwave oven, and the sample was processed in a three-step procedure. First, the temperature reached 165 in 8 mins and hold for 5 mins, then it reached 185 in 5 mins and hold for 20 mins, and finally cooled down in 15 mins. Then place the Teflon digestion vessels at 160°C to volatilize the acid to 1 mL. The solution was transferred to a 50 mL volumetric flask and diluted to 50 mL with ultrapure water, and 15 mL of the solution was taken for determination. Teflon digestion vessels were previously washed in HNO_3 to avoid cross-contamination.

2.3 Determination by ICP-OES and ICP-MS

Due to different detection limits of the instrument, the concentrations of 9 elements (Al, Cd, Co, Cr, Fe, Mo, Ni, Pb, and Zn) in the *PN* samples were determined by using ICP-OES (Optima 8000, Perkin Elmer, USA). The other 8 elements (B, Ca, Cu, K, Na, Mg, Mn, and P) were determined by using ICP-MS (ELAN, DRC-e, Perkin Elmer, USA). The detection wavelength of each element as shown in Table 2.

2.4 Statistical Analysis

All experimental data were managed using Microsoft Excel 2016. HCA was used for statistical analysis to examine the relationship among the four *Panax* species based on the contents of elements. PCA was used to analyze the main inorganic elements in *PN*. HCA and PCA were used all based on SPSS statistics 17.0 software.

3. Results and discussion

3.1 Dynamics of elements during the three-year growth process of *P. notoginseng*

The elemental contents of *Panax notoginseng* in different periods have been determined using ICP-OES and ICP-MS, and the results were listed in Table 3. Ca, Mg, K, P were macroelements in plants, which had a high content in plants (Fig. 1). The content of K in the root of *PN* reached the maximum in August of the second year. In April, May, and October of each year, the potassium accumulation was lower than other periods, so we can consider increasing the amount of potassium fertilizer in April, May, and October. Ca, Mg, P were highest in *PN* in June of the second year. Overall, there was no significant difference in the accumulation of Mg and P in *PN*. Among these macroelements, phosphorus was beneficial to plant growth and development, calcium can ensure cell life activity, while magnesium was involved in plant photosynthesis and was an activator or component of many enzymes. Therefore, increasing the application of Ca fertilizer might be beneficial to the growth of *PN*, except for April, May, and June.

Microelements in traditional Chinese medicine play an important role in disease resistance, cancer prevention, longevity and other aspects (Zhao et al., 2013), including Fe, Cu, Zn, Mn, Mo, B, Na, and Ni. The accumulation of trace elements was higher during the growing period of *PN*, but B reached its peak in December of the third year. Fe was the highest content of the eight measured trace elements, which was an essential element for plants. The presence of Fe was a necessary condition for chlorophyll biosynthesis. The accumulation of Fe was higher during the growing period of *PN*. Therefore, the amount of Fe fertilizer can be increased in August, September, and October of the first two years in actual production.

In addition to macroelements and trace elements, there were also beneficial elements, such as Al. Al was harmful to most plants, but it can promote growth in some plants. The presence of Al in the soil can improve the growth of plants, regulate the absorption of certain elements by plants and stimulate certain physiological metabolic processes. The change of aluminum accumulation in *PN* in different periods was consistent with the growth rule of *PN*. *PN* above two years included two growth peaks, namely the peak of vegetative growth from April to June and the peak of reproductive growth from August to October. The accumulation of Al in *PN* was higher during the growth peak, and was the highest in May of the second year, reaching

6694.32 $\mu\text{g/g}$ (Fig. 2). After the growth period, the accumulation showed a significant downward trend. The accumulation of Al in the *PN* in November was the lowest at 307.66 $\mu\text{g/g}$.

With the modernization of Chinese traditional medicine, the safety of Chinese traditional medicine becomes a growing emphasis and the toxic heavy metal pollution is one of the main problems (Shi et al., 2008). Heavy metals can affect the body's metabolism, thereby reducing the body's immunity and increasing the risk of cancer (Zhang et al., 2012). According to the *Green Industry Standard for the Import and Export of Medicinal Plants and Preparations* for heavy metals in Chinese medicinal materials, the highest level of Cd, Cu, Pb, As, and Hg is 0.3, 20, 5.0, 2.0, and 0.2 mg/kg, separately. The result showed that no matter which period, the content of Cd in *PN* exceeded the limit. The Pb content of *PN* in July and August of the first year exceeded the standard, and the Cu content in April of the third year exceeded the standard. *PN* is generally harvested in October of the third year. Except for Cd, the heavy metal content measured in *PN* reached the national standard in October of the third year. Therefore, when selecting the soil for planting *PN*, it is necessary to pay attention to whether there is heavy metal pollution in the soil, especially cadmium pollution, to avoid the problem of excessive heavy metal content in *PN* due to heavy metal pollution in the soil.

3.2 Comparison of element content in leaves and roots of *Panax notoginseng*

In order to compare the differences in the content of elements in different parts of *Panax notoginseng*, the contents of 17 elements in the leaves and roots of *PN* in October of the third year were determined (Fig. 3). The contents of B, Ca, K, Mg, Na, Mn, Mo, Pb, Zn in leaves were significantly higher than those in the roots, while the Cd content in leaves was significantly lower than that in the roots. The Ca content in the root was only 1.86%, while it was as high as 11.90% in the leaves. This showed that Ca may play a greater role in the leaves than in the roots. K was twice as much in leaves as in roots. Ca^{2+} , K^{+} together as the corresponding ions of H^{+} participated in oxidative phosphorylation, indicating that oxidative phosphorylation in leaves might be more active.

3.3 Differences of element content between *PN* and other *Panax* species

The elements in *Panax bipinnatifidus*, *Panax stipuleanatus* and *Panax vietnamensis*, which also belong to *Panax*, were determined and compared with those in *P. notoginseng*, and the results were shown in Table 4. To visually demonstrate the distribution rules of the elemental contents, a line graph of the determination results was shown in Fig. 4. As viewed from this figure, the differences of the elemental content could be seen clearly among the four species, and the content of an individual element was always maintained within a certain range except Al, Fe and Na. The element content of *P. vietnamensis* was generally higher than that of other species, except that the B content was the lowest among the four species. The Fe content in *P. vietnamensis* was significantly higher than that in other species, which may be related to its growing environment. Although they were different in origin, soil and environment, the four species had a certain balance in the absorption of various elements. There were differences in the elemental peak types of the four species, providing a basis for constructing elemental fingerprints of each species, which supported the application of inorganic elemental fingerprint for the determination of *Panax*.

Based on the element content, the HCA was used to calculate the kinship of the four *Panax* species. Similarities among the species were calculated using the Statistics software SPSS. The result of HCA was shown in Fig. 5. The results showed that the four species could be divided into two clusters: *P. vietnamensis* and *P. stipuleanatus* were in cluster 1, and *P. notoginseng* and *P. bipinnatifidus* were in cluster 2. According to the clustering distance, the *P. vietnamensis* and *P. stipuleanatus* exhibited more similarity in elements than the other two species.

3.4 PCA analysis

PCA can use fewer factors to explain more variables (Lin et al., 2005; Xu and Shao, 2006). According to the PCA results, the first four principal components (PC1-4), with eigenvalues >1 , explained 81.01% of the total variability among the 17 variables in the original data, where PC1, PC2, PC3, and PC4 contributed

43.24%, 16.46%, 13.07%, and 8.24% of the total variance, respectively (Table 5). Thus, the result showed that a four-factor model could explain 81.01% of the test data. According to vectors listed in Table 5, the Al, Co, Fe, Ni, Pb, Cu, and Cr weighed highly in PC1; K, B, Mg, and Na loaded highly in PC2; Mn and P weighed highly in PC3; Ca was the main feature element in PC4.

From the three-dimensional diagram of the composition of the first, second and third principal components (Fig. 6), it can be seen that there was a significant separation between *Panax* samples. *P. vietnamensis* and *P. stipuleanatus* were significantly separated from *P. notoginseng* and *P. bipinnatifidus*, which were consistent with HCA analysis (Fig. 7). However, the results of PCA and HCA indicated that it is hard to distinguish *P. notoginseng* from different periods based on element content. The accumulation and changes of elements during the growth of *P. notoginseng* were irregular. Thus, it is difficult to distinguish *P. notoginseng* from different periods by element content.

4. Conclusion

There is currently no research on the accumulation of elements in different growth periods of *Panax notoginseng*. The contents of 17 elements in different growth periods of *P. notoginseng* were measured by ICP-OES and ICP-MS, and there was a certain dynamic change in the accumulation of each element. Therefore, according to the accumulation of elements in different periods of *PN*, scientific fertilization can be carried out to ensure a balanced supply of nutrients and improve the quality and yield of *PN*. Increasing the amount of K fertilizer in April, May, and October, the amount of Ca fertilizer except for April, May and June, and the amount of Fe and Al fertilizer in August, September, and October might be beneficial to the growth of *PN*.

The content of elements in leaves and roots also differed in the same period. The contents of B, Ca, K, Mg, Na, Mn, Mo, Pb, Zn in leaves were significantly higher than those in the roots. The root of *PN* is often used as a medicinal material. The Cd content in the root samples collected in this study exceeded the standard, which may be the serious pollution of cadmium in the soil of the growing area. Therefore, it is necessary to choose the appropriate area for the cultivation of *P. notoginseng* to ensure that the content of heavy metals in the root meets national standards.

By comparing with the content of elements in other *Panax* species, it was found that the *P. vietnamensis* and *P. stipuleanatus* exhibited more similar in elements than the *P. notoginseng* and *P. bipinnatifidus*. In addition, we can distinguish different *Panax* species based on element content. However, it was difficult to distinguish *P. notoginseng* from different periods by element content due to its irregular change of elements.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1 Sample information

Number	Species	Period	Position
1	<i>Panax notoginseng</i>	Y1M6	root
2		Y1M7	
3		Y1M8	
4		Y1M9	
5		Y1M10	
6		Y2M4	
7		Y2M5	
8		Y2M6	
9		Y2M7	
10		Y2M8	
11		Y2M9	
12		Y2M10	
13		Y3M4	
14		Y3M5	
15		Y3M6	
16		Y3M7	
17		Y3M8	
18		Y3M9	
19		Y3M10	
20		Y3M11	
21		Y3M12	
22	2 year	leaf	
23	2 year		
24	3 year		
25	<i>Panax vietnamensis</i>	-	root
26	<i>Panax stipuleanatus</i>	-	
27	<i>Panax bipinnatifidus</i>	-	

Notes: Y1M6 means *Panax notoginseng* June of the first year and so on.

Table 2 Analytical wavelength for each element

Element	Wavelength (nm)	Element	Wavelength (nm)
Al	396.152	K	766.490
B	249.677	Na	589.592
Ca	317.933	Mg	285.213
Cd	214.439	Mn	257.610
Co	228.615	Mo	202.032
Cr	283.563	Ni	231.604
Cu	327.393	P	213.617
Fe	371.994	Pb	220.353
Zn	202.548		

Table 3 The elemental contents ($\mu\text{g/g}$) of *Panax notoginseng* in different periods

Period	Elements ($\mu\text{g/g}$)					
	Al	B	Ca	Cd	Co	Cr
Y1M6	832.66	6.50	1433.25	1.17	0.68	0.79
Y1M7	4449.32	1.83	1307.08	1.87	2.38	4.26
Y1M8	2532.66	7.00	1898.42	2.43	1.96	2.69
Y1M9	1982.66	8.33	1425.92	1.23	1.23	2.37
Y1M10	2332.66	5.17	1274.42	0.85	0.84	2.71
Y2M4	3865.99	8.00	4273.42	1.72	1.28	7.77
Y2M5	6699.32	2.50	2890.08	1.49	2.14	19.12
Y2M6	4565.99	11.17	5260.08	2.17	2.04	7.14
Y2M7	2465.99	10.33	2018.42	2.05	1.30	3.94
Y2M8	1427.66	10.50	1981.75	1.87	0.86	6.09
Y2M9	4132.66	7.50	1925.08	3.27	1.51	7.39
Y2M10	814.32	11.00	1422.42	0.95	0.36	2.21
Y3M4	5232.66	1.83	2745.08	3.15	1.71	24.96
Y3M5	5915.99	2.83	2751.75	1.87	2.16	21.79
Y3M6	5499.32	6.83	3483.42	2.57	2.16	15.19
Y3M7	2649.32	5.83	2106.75	1.20	1.15	7.49
Y3M8	5232.66	2.50	2135.08	1.45	1.37	13.57
Y3M9	5682.66	2.67	1791.75	0.81	1.38	15.49
Y3M10	1899.32	6.33	1860.08	0.75	0.67	6.74
Y3M11	307.66	8.50	1420.92	0.35	0.16	3.79
Y3M12	499.32	12.33	1398.08	0.62	0.27	2.36

Period	Elements							
	Mg	Na	Mn	Mo	Ni	P	Pb	Zn
Y1M6	1773.58	57.33	374.67	0.0065	4.59	2149.17	2.82	16.08
Y1M7	1551.42	37.83	995.67	0.1218	7.12	1357.67	5.42	22.08
Y1M8	1828.58	83.83	1241.67	0.1040	6.95	1932.50	5.46	42.75
Y1M9	1552.42	52.33	543.17	0.0745	5.22	1541.33	2.86	24.00
Y1M10	1425.42	41.17	382.83	0.0982	4.99	1501.33	2.67	26.58
Y2M4	2745.25	312.17	59.17	0.3717	12.00	1437.50	3.32	41.25
Y2M5	2791.92	109.83	81.33	0.2050	12.75	1246.00	4.66	32.83
Y2M6	3425.25	1278.83	88.00	0.2417	6.85	3014.17	4.16	29.75
Y2M7	2540.25	51.50	110.50	0.0945	5.42	2234.17	2.59	19.75
Y2M8	1885.25	52.00	77.00	0.0398	7.79	2140.83	2.06	19.67
Y2M9	1860.25	48.17	143.83	0.1153	7.49	2557.50	3.84	27.75
Y2M10	1654.25	23.50	25.50	0.0953	3.42	1817.50	0.92	12.50
Y3M4	2243.58	135.17	108.33	0.2967	8.25	1221.50	3.81	35.58
Y3M5	1950.25	73.50	136.67	1.7500	14.85	1455.00	4.27	25.00
Y3M6	2303.58	80.67	159.50	0.3800	9.00	1852.50	4.59	40.83
Y3M7	1826.92	45.33	105.83	0.1375	4.85	1684.17	2.22	29.58
Y3M8	1793.58	45.17	105.17	0.2250	6.90	1685.83	3.27	33.17
Y3M9	1533.25	53.17	78.17	0.2450	7.19	1925.83	3.31	28.33
Y3M10	1500.58	25.33	40.17	0.0643	4.12	1659.17	1.37	20.08
Y3M11	1349.92	14.83	13.00	0.0054	1.51	1754.17	0.29	6.56
Y3M12	1555.75	18.67	17.17	0.0084	1.94	2014.17	0.64	9.80

Table 4 The elemental contents ($\mu\text{g/g}$) of different *Panax* species

Elements	<i>P. stipuleanatus</i>	<i>P. vietnamensis</i>	<i>P. bipinnatifidus</i>	<i>P. notoginseng</i>
Al	689.32	2532.66	347.66	1832.66
B	9.83	2.00	6.00	4.50
Ca	11488.42	7573.42	8631.75	5050.08
Cd	0.50	0.55	0.11	0.20
Co	0.33	0.46	0.22	0.17
Cr	5.62	10.06	1.66	6.99
Cu	5.00	10.67	6.50	5.42
Fe	396.01	2151.35	317.26	215.85
K	22609.25	22775.92	15659.25	11970.08
Mg	2993.58	2998.58	1913.58	1668.58
Na	230.67	127.67	73.33	26.11
Mn	29.50	51.33	27.67	20.33
Mo	0.0101	0.1252	0.3200	0.0190
Ni	2.10	5.20	1.45	1.88
P	1000.17	1081.00	1104.50	1297.50
Pb	1.23	2.46	0.73	1.76
Zn	20.58	29.25	15.58	12.58

Table 5 Results of Principal Component Analysis

Items	Principal components	Principal components	Principal components	Principal components
	1	2	3	4
Al	0.956	-0.106	-0.066	-0.040
B	-0.371	0.688	0.341	-0.260
Ca	-0.131	0.427	-0.733	0.429
Cd	0.785	0.194	0.391	-0.042
Co	0.918	-0.027	0.265	0.165
Cr	0.804	-0.177	-0.397	-0.249
Cu	0.814	0.014	0.010	-0.392
K	0.126	0.769	-0.109	0.078
Fe	0.935	-0.020	-0.218	-0.117
Mg	0.463	0.696	-0.330	0.199
Na	0.298	0.673	-0.083	0.180
Mn	0.152	-0.243	0.653	0.643
Mo	0.472	-0.318	-0.380	-0.167
Ni	0.914	-0.065	-0.020	-0.093
P	0.306	0.595	0.588	-0.343
Pb	0.838	-0.087	0.280	0.340
Zn	0.667	-0.073	-0.101	0.358
Variance (%)	43.243	16.458	13.066	8.241
Cumulative variance (%)	43.243	59.701	72.768	81.009

Fig. 1 Macroelements of *Panax notoginseng* in different periods

Fig. 2 The content of Al of *Panax notoginseng* in different periods

Fig. 3 Content of elements in leaves and roots of *Panax notoginseng*

Fig. 4 The elemental contents of different *Panax* species.

Fig. 5 HCA dendrogram of four species of *Panax*

Fig. 6 PCA scores of 27 samples of *Panax* based on 17 elements

Fig. 7 HCA dendrogram of 27 samples of *Panax*







