

Full Length Research Paper

# ICP-MS determination of trace elements of different growth time in the leaves of *Crataegus pinnatifida* Bge. var. *major*

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Accepted 4 July, 2011

**A rapid inductively coupled plasma mass spectrometry (ICP-MS) with semiquantitative modes of analysis using Li, Y, Ce, Tl and Co for external calibration of the equipment was developed to simultaneously determine 53 trace elements in the leaves of *Crataegus pinnatifida* Bge. var. *major*. Samples were analyzed directly following an acid digestion with 5 ml of conc. HNO<sub>3</sub> by the microwave digestion system. The analytical data were obtained over a wide range from 30418.701 ng g<sup>-1</sup> of K to 0.759 pg g<sup>-1</sup> of Ta. The results indicated that the quality of the leaves could be effectively evaluated according to the distributions of the trace element in the leaves from different growth stages.**

**Key words:** Inductively coupled plasma mass spectrometry (ICP-MS), trace elements, leaves of *Crataegus pinnatifida* Bge. var. *major*.

## INTRODUCTION

The leaves of *Crataegus pinnatifida* Bge. var. *major* are recorded in the Chinese Pharmacopoeia as a well-known traditional Chinese medicine for the treatment of qi-stagnancy and blood stasis, chest distress, palpitation and loss of memory, dizziness and tinnitus (PRC, 2010). Till now, most previous studies concentrated mainly on pharmacological actions (Ying et al., 2008a; Ying et al., 2008b), pharmacokinetic (Ying et al., 2007a; Ying et al., 2007b) and the chromatographic analysis (Ying et al., 2009) of polyphenols in the leaves of *C. pinnatifida* Bge. var. *major*. To our knowledge, there are no published reports of trace elements in the leaves of *C. pinnatifida* Bge. var. *major* from different growth stages. It is reported that some trace elements have been recognized to be necessary for plant growth and the concentrations depend on the plant species, their ages and the growth

conditions in the environment (Hokura et al., 2000). Therefore, it is necessary to develop an assay to fully evaluate the trace element distribution in the leaves of *C. pinnatifida* Bge. var. *major*.

To analysis of trace element, many methods such as the atomic absorption spectrometry (AAS) (Ozmen et al., 2006) and inductively coupled plasma atomic emission spectrometry (ICP-AES) (Paredes et al., 2006) were widely used. However, AAS with narrow linear range only determines a single element for each run and ICP-AES does not enable the removal of spectral interference. Inductively coupled plasma mass spectrometry (ICP-MS) has become a widely accepted technique for the determination of trace elements during the past two decades, compared with the traditional inorganic analytical technique, ICP-MS offers many advantages such as wider linear range, lesser interference (Huang et al., 2006) and was used to the analysis of essential elements and well-recognized potentially toxic metals (Pappas et al., 2006). As we all know, multielement profiling analysis is significantly required in modern science for a comprehensive understanding of the biological and physiological functions in plants. ICP-MS

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**Table 1.** Instrumental operating conditions for ICP-MS.

ICP system	
Carrier gas	1.14 L/min
RF power	1300 W
Atomizer chamber	2°C
Sample uptake rate	0.1 rps
Points per spectral peak	6
Number of replicate	3
Sampling depth	8.2 mm
Mass spectrometer	
Sampling cone	Nickel, -96.2 V
Skimmer cone	Nickel, -22 V
Vacuum	$5 \times 10^{-7}$ Mba
Mass range	2 to 260 amu
Total acquisition	181 s

with semiquantitative analysis has proven to be a powerful tool for fast screening of unknown samples (Chen et al., 2008). The measured results of semiquantitative analysis are equal to or even better than those obtained by quantitative analysis (Laborda et al., 2001).

The aim of this study is to develop a rapid ICP-MS method with a semiquantitative analysis mode to investigate the trace element distribution in the leaves of *C. pinnatifida* Bge. var. *major*, thereby, which will be exploited and utilized reasonably.

## EXPERIMENTAL

### Instrument

An Agilent 7500a ICP-MS (Agilent Technologies Co., Ltd, USA) was used for the determination of trace element with a semiquantitative analysis. Agilent 7500 ICP-MS ChemStation software was used for data acquisition. A MDS-6 digester/extractor including a microwaver and PTFE vessels was from Shanghai Xinyi Microwave Chemical scientific and technology Co., Ltd (Shanghai, P. R. China).

The instrument was optimized daily in terms of sensitivity (Li, Y, and Tl), level of oxide (CeO/Ce) and doubly charged ion (Ce<sup>+2</sup>/Ce) using a tuning solution containing 10<sup>-9</sup> g/ml of Li, Y, Tl, Ce and Co in 2% HNO<sub>3</sub> to meet the demands of the trace element determination. The operating conditions of ICP-MS instrument were summarized in Table 1.

### Chemicals

Ultrapure water was prepared with a Milli-Q deionization unit (Millipore, Bedford, MA, USA). Nitric acid used for sample digestion was of high-purity grade and purchased from Kermel Chemical Reagent Co., Ltd (Tianjin, P.R. China). Wash-Nitric Acid Blank: Part# G 1820-60258 (5% HNO<sub>3</sub>), Wash-water blank: Part# G 1820-60259, tuning solution of MS optimization: 10<sup>-9</sup> g/ml of Li, Y, Ce, Tl and Co (2% HNO<sub>3</sub>) (Part# G5184-3566) was used to perform external calibration, and the internal standard solutions including four elements of 10<sup>-9</sup> g/ml of Sc, Ge, In and Bi (5% HNO<sub>3</sub>) (Part# G

5183-4680) used to reduce matrix effect and compensate for instrument drift during the analysis were purchased from Agilent (NJ, USA) and diluted to approximately 8 ng/g by 5% HNO<sub>3</sub> before the experiment.

### Sample collection

Dried leaves of *C. pinnatifida* Bge. var. *major* were collected weekly in Liaozhong from May 20 to October 22, 2010. Voucher specimens (20101023) are maintained at Liaoning University of Traditional Chinese Medicine, China.

### Microwave digestion

To the 50 ml closed PTFE vessel, 0.35 g of the dried and powdered leaves of *C. pinnatifida* Bge. var. *major* and 5 ml of conc. HNO<sub>3</sub> was added and then digested by microwave digestion system. During this study, the microwave pressure and time were examined under the condition of microwave power of 600 W for every step. The optimized digestion conditions were a four-step program for 6 vessels including step 1 to 3 (Pressures were 0.3, 0.6 and 1.0 MPa for 4 min, respectively) and step 4 (Pressure was set at a maximum pressure of 1.5 MPa for 10 min). The microwave controller automatically shuts down power when the pressure in this control vessel reaches a preset maximum. The digested solutions were stored in a refrigerator at 4°C and subjected to analysis within 48 h after dilution.

### Blank determination

To evaluate the possible contributions for the blank of ultrapure water and nitric acid, the test of blank solution was performed before analysis of the leaves of *C. pinnatifida* Bge. var. *major* in each analytical session. The blank solutions were prepared together with the samples using the ultrapure water and nitric acid for the dilution steps. For the obtained values for the blank solution were not negligible, therefore, all the blank contributions were subtracted from the trace element concentrations found in the samples via the software of ICP-MS.

### Stability

The final digestion solutions of the leaves of *C. pinnatifida* Bge. var. *major* were kept for 0, 6, 12, 18, 24 and 48 h at 4°C in a refrigerator, respectively, then, introduced to ICP-MS for analysis. In this study, the 8 elements including Pt, Dy, Zr, Cu, Zn, Fe, Si and Ca from low to high concentration were selected for the investigation of the stability.

## RESULTS AND DISCUSSION

### Sample preparation

Weighing accurately 0.35 g ( $\pm 0.1$  mg) of the dried and powdered leaves of *C. pinnatifida* Bge. var. *major*. Adding 5 ml of conc. HNO<sub>3</sub>, the samples were digested for 22 min under the optimized digestion conditions. Then, the solution was transferred into a known weight of PET tube and diluted to about 10 ml with ultrapure water, subsequently, weighed. The 2.5 ml of the diluted solution was transferred to known weight of 50 ml vial with

**Table 2.** Stabilities of the diluted digestion sample.

Time (h)	Ca (ng/g)	Si (ng/g)	Fe (ng/g)	Zn (ng/g)	Cu (ng/g)	Zr (pg/g)	Dy (pg/g)	Pt (pg/g)
0	21469.0	1016.0	445.1	39.3	1.65	102.1	23.0	3.40
6	22396.4	1341.6	506.8	50.1	1.62	80.5	23.9	3.39
12	19740.0	1347.8	436.3	40.7	1.76	104.8	21.0	3.52
18	20770.1	1053.6	342.8	31.4	1.80	95.4	22.7	3.19
24	18604.7	1059.1	454.2	38.9	1.75	107.6	20.9	3.46
48	25610.9	1162.8	454.9	54.6	1.74	128.5	24.0	3.53
–	21431.9±	1163.5±	440.0±	42.5±	1.72±	103.2±	22.6±	3.42±
X ± SD	2436.8	148.6	53.6	8.4	0.068	15.8	1.4	0.12
RSD (%)	11.4	12.8	12.2	19.7	3.9	15.3	6.0	3.6

22.5 ml of ultrapure water and then weighed. Finally, samples were approximately diluted by 100-fold with ultrapure water using weight/weight preparation throughout the process of sample dilution, meanwhile, the matrix effect can be also alleviated by dilution of the digested solution.

For an ICP-MS measurement, nitric acid is used as a reagent blank solution and a washing/rinse solution, which shows the lowest background levels next to the pure water and the simplest spectra compared with hydrofluoric, perchloric and sulfuric acid (Lee et al., 2003). In this study, nitric acid was used because of its oxidizing ability in practical analysis. All of the plant samples were decomposed with 5 ml of conc. HNO<sub>3</sub>, the lowest volume for sample digestion in the microwave digestion system.

### Sample stability

The results of the stability study involving 8 elements from low to high concentration were presented in Table 2. The relative standard deviations (RSDs) were below 19.7%, which confirmed the high stability of the diluted digestion

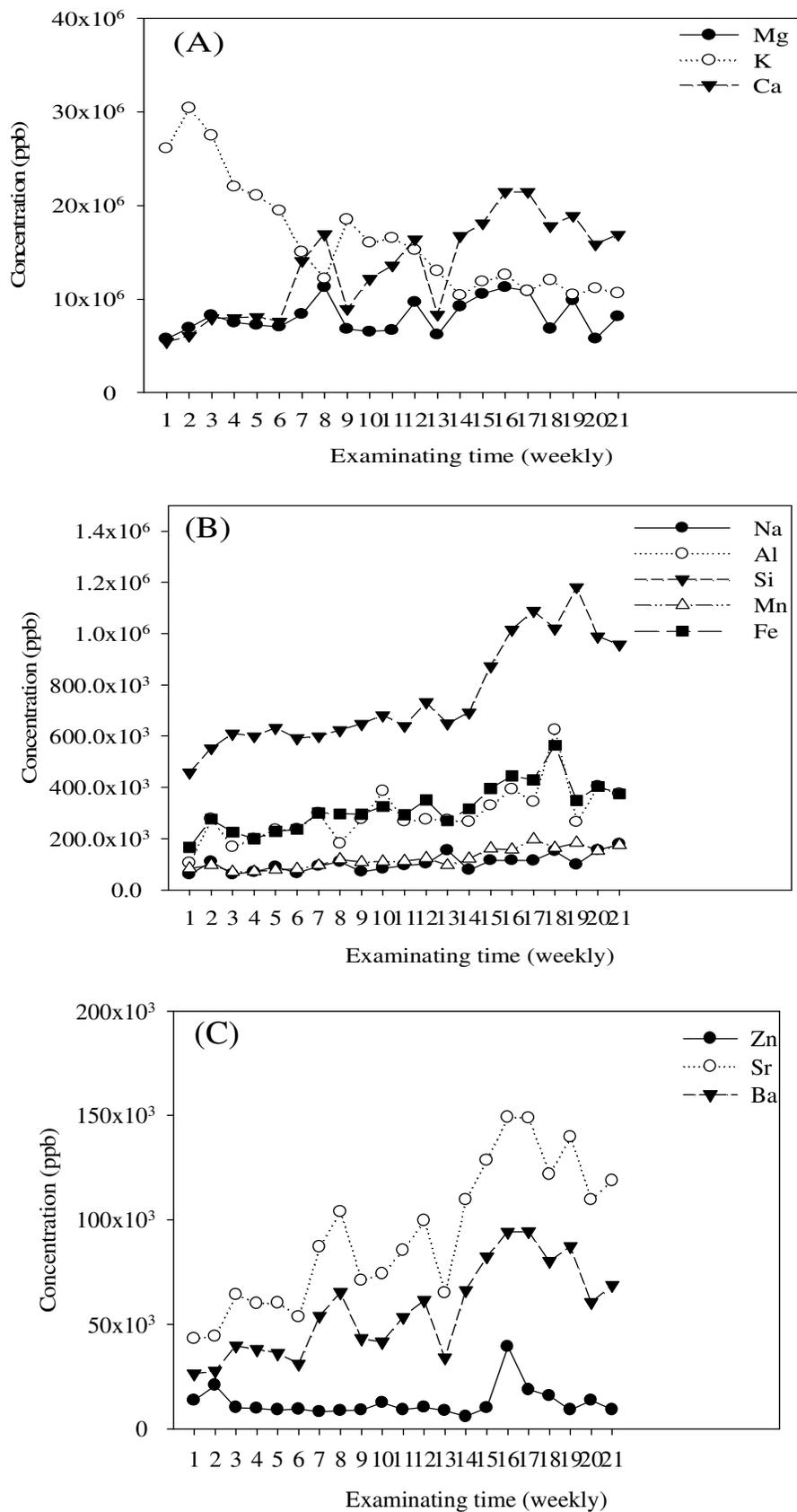
solution within 48 h under the semiquantitative analysis mode.

### Sample analysis

Wash-nitric acid blank and wash-water blank were used to rinse the system and flow path, respectively, before the analysis. Then, tuning solution including 10<sup>-9</sup> g/ml of Li, Y, Ce, Tl and Co was utilized to modulate the conditions of the system. The preconcentration for the digested solution should be done for trace element determination, such as evaporation (Lee et al., 2000) and solvent extraction (Anbar et al., 1997), however, which is infeasible because, possible contaminations from the experimental environment. Therefore, the sample was directly introduced by a peristaltic pump at 0.3 ml/min flow rate following the sample diluted with 5% HNO<sub>3</sub>. The reference internal standard of approximately 8 ng/g of Sc, Ge, In and Bi was added on-line as the reference solution to alleviate matrix effect and compensate for signal drift in each individual run on the ICP-MS. The trace elements, of which the concentration levels of most trace elements

down to ppt level and under the interferences of oxide (Ti, Ni, Se, Eu and Cr), argide (Hf, Mo and Lu), hydride (La and Cr) and dimer (Yb), were not included. Finally, the total number of the analysis element was 53. The total analysis time was 181 seconds. Figure 1 showed that the trace elements varied in the leaves from different growth stages. The high concentrations of K, Ca and Mg were found in Figure 1A-D and that of Ca and Rb decreased significantly in the different growth stages. Na, Al, Si, Mn, Fe, Zn, Sr and Ba were over the concentration range from 1182.3 to 5.771 ng/g in Figure 1b and C, from which we observed that these trace elements except that Zn varied insignificantly, increased in the whole growth stage of the plant. The analytical data indicated that nearly all the trace elements in Figure 1a to d were the main elements of the leaves, in which the highest content of Ca was 1182.3 ng/g, and that Si varied significantly and gradually increased along with the growth of the plant, therefore, can be taken as the index of the growth of the leaves.

Conversely, the content of Ca decreased gradually from high to low, suggesting that Ca can be also used as the index for the reasonable exploitation and utilization of the leaves. All



**Figure 1.** (A-J) Trendgraphs of the concentration of 53 elements in the leaves of *Crataegus pinnatifida* Bge. var. major

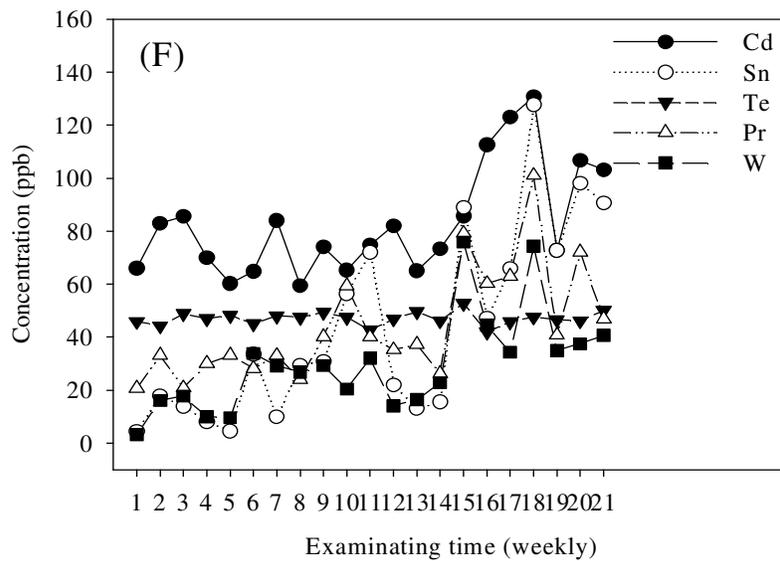
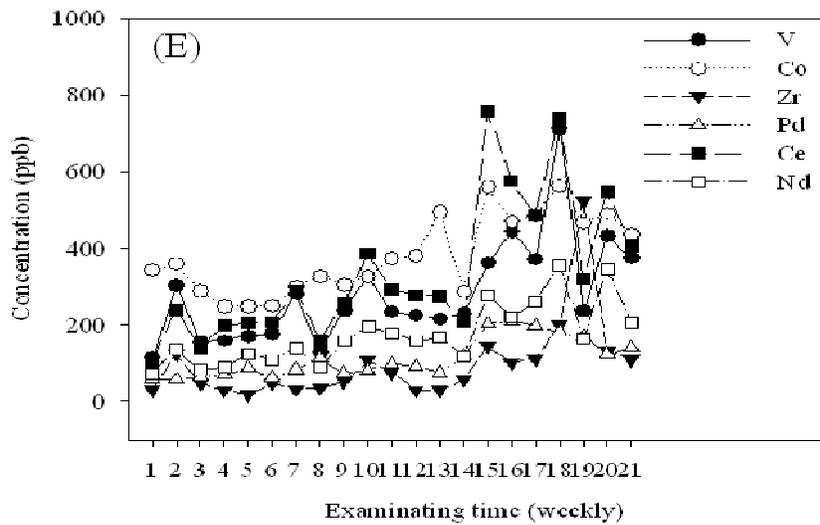
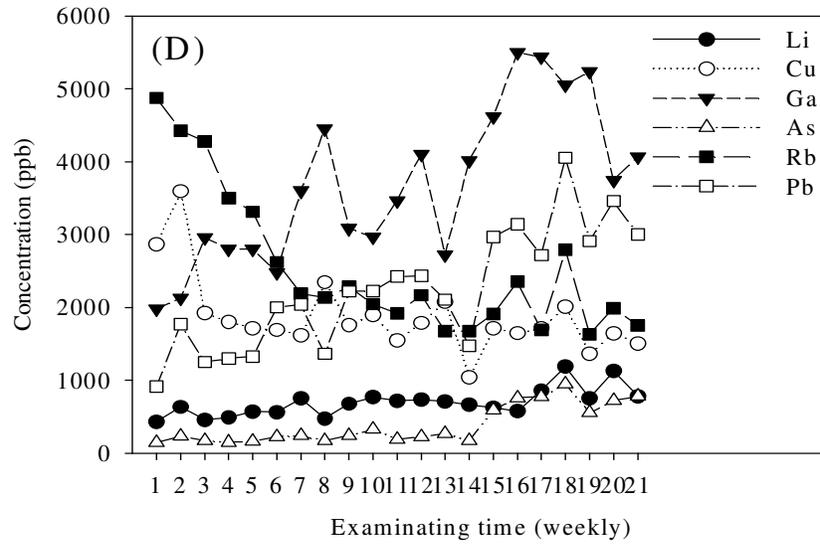


Figure 1. Contd.

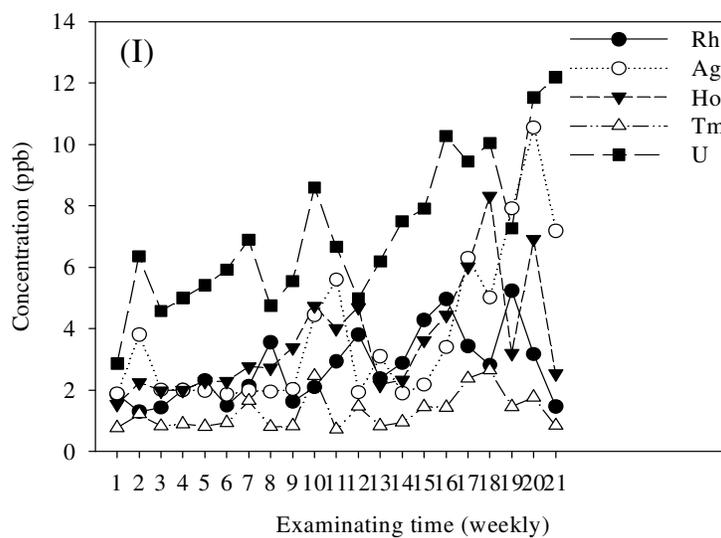
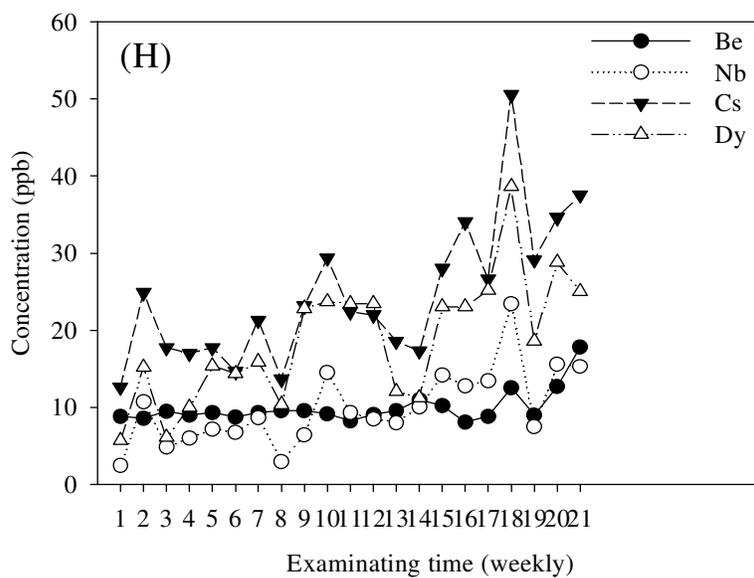
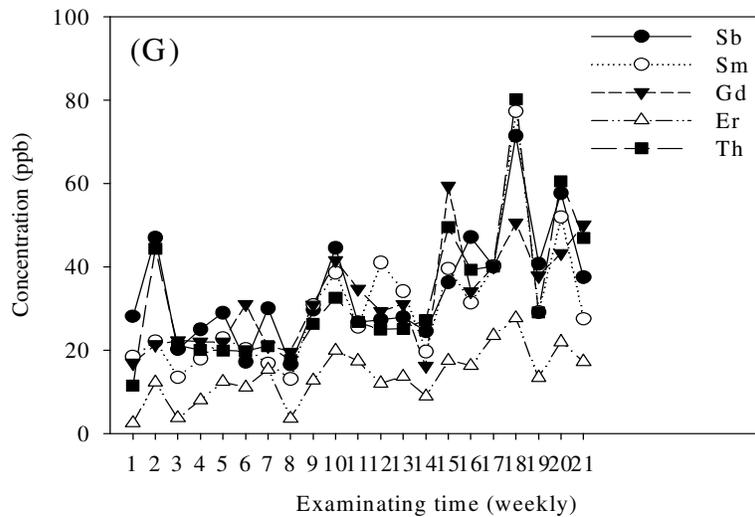


Figure 1. Contd.

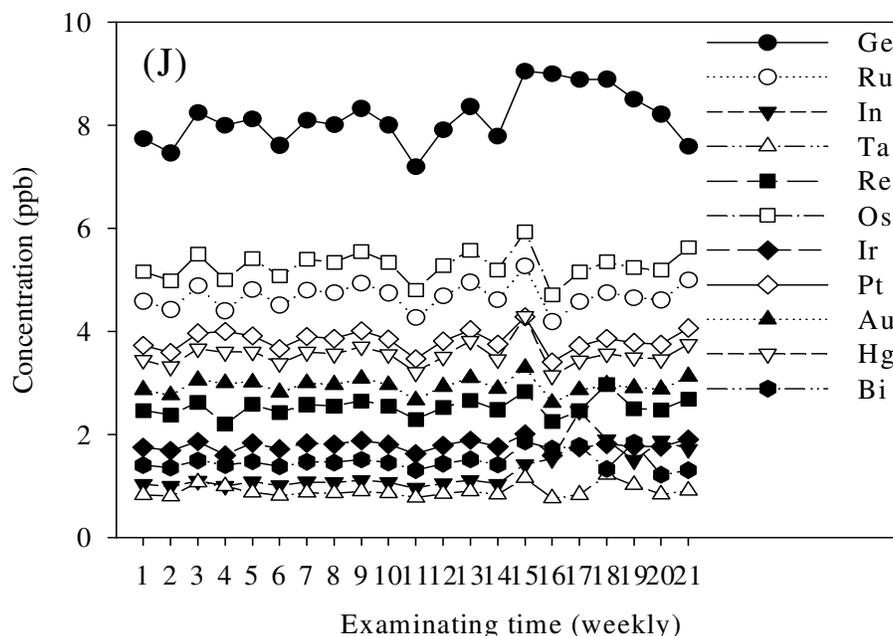


Figure 1. Contd.

trace elements in Figure 1d increased in different growth stage except that concentration of Te varied insignificantly. Figure 1J indicated that the concentrations of the trace elements of Ge, Ru, In, Ta, Re, Os, Ir, Pt, Au, Hg and Bi were very low but with comparatively stable content in the whole growth stage.

## Conclusions

The leaves of *C. pinnatifida* Bge. var. *major* from different growth stages were analyzed by ICP-MS method using the semiquantitative analysis mode which gives us very useful information about the 53 element determined in this paper and the distribution of the trace elements must be considered when we exploit and utilize the leaves of *C. pinnatifida* Bge. var. *major*.

## ACKNOWLEDGEMENT

This present work was supported by the Natural Science Foundation of Liaoning Province (20072073), China.

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