Biogeochemical effect of karst soil on the element contents in the leaves of two species of *Flos Lonicerae*

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ABSTRACT

In order to study the characteristics of element values in the cell of plants and soils and their relationships that would help to evaluate the biogeochemical effect of soil on the element contents in the cells of plants in the same environment of Southwestern China, the soil samples were collected for analysis and the weight and atom percentage of elements (WT% and AT%) in the leaves of two different species of *Flos Lonicerae* were analyzed by the electron probe (EDX-9100). The results of soil analysis show that the nutrient element contents in the soil are determined by the liable content of elements, which were arranged as: Ca > Mg > Cu > Mn > K > Na > P > B > SiO_2 > Zn > Fe, even though the total element values in soil of different horizons were arranged as: SiO_2 > Fe > Ca > Mg > K > Na > Mn > P > Zn > B > Cu. It indicates that karst environment is composed of soluble calcium-rich rock and soil scarcity. Moreover, the migratory velocity and availability of elements are also determined by their coefficient variability. According to statistical results, Ca, Mn and P in the soil have high coefficient variability, reflecting its background of karst soil. Based on the electron probe, it is shown that the content of Ca is higher while Mg is the lowest in the cell of two different species of *Flos Lonicerae* ($r = -0.156$, $P < 0.05$) and the content of P in the cell is inferior to Ca ($r = 0.868$, $P < 0.01$). By studying the relationship of soil and the plants, it can be seen that these results are probably caused by the characteristics of local biogeochemistry. Ca is mainly absorbed by plant with the help of transpiration and accumulated in the cell, and thus becomes a predominant element in the cell. The accumulation of Ca in cell of plant will affect the absorbency and the content of other elements in the cell of *Flos Lonicerae*, resulting in the different contents of other elements in the *Flos Lonicerae* through synergistic and antagonistic action, followed by the affected quality and officinal value of the *Flos Lonicerae*.

Keywords: *Flos Lonicerae*; biogeochemistry; element contents; electron probe; karst

Flos Lonicerae (Jinyinhua in Chinese), the dried buds of several species of the genus *Lonicera* (*Caprifoliaceae*), is a commonly used traditional Chinese medicine (TCM) herb. It has been used for centuries in TCM practice for the treatment of sores, carbuncles, furuncles, swelling and affections caused by exopathogenic wind-heat or epidemic febrile diseases at the early stage (Editorial Pharmacopoeia Commission of People's Republic of China 2000). *Flos Lonicerae* has these functions due to its bioactive components, i.e. chlorogenic acid and its analogues, iridoid glucosides, flavonoids and triterpenoid saponins, which are isolated by chemical and pharmacological investigations (Li et al. 2003, Wu 2007). According to the documents, there are about 47 species of *Loniceras*

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documented as the sources of *Flos Lonicerae* in China Pharmacopeia (2000 edition), i.e. *L. japonica* Thunb., *L. confusa* and *L. daystyla*. In addition, *Flos Lonicerae* mainly distributes in Southwestern China and is planted to harness rocky desertification due to its adaptability to the rock-desertification environment in karst areas that are Ca-rich and have deficit in water and soil (Yuan 2001).

In view of its beneficial effects, previous quality control and evaluation of *Flos Lonicerae* were usually concerned with chlorogenic acid because of its high content (no less than 1.8%) and antipyretic property (Li et al. 2003, Chai et al. 2005, Liao et al. 2005). However, little research on the biogeochemical effect on the element contents of *Flos Lonicerae* has been done. The quality of *Flos Lonicerae* is affected by soil environment, which not only provides the nutrition that the plant need for growth, but also can determine the elements content. Hereby, it is important to study the plant-soil ecosystem.

MATERIAL AND METHODS

Plant material and growth conditions. Adult leaves of *Lonicera japonica* Thunb. and *Lonicera confuse* and soil samples prepared for this study were collected from the Nongla Karst Experimental Site, Institute of Karst Geology, Chinese Academy of Geological Sciences.

For the element analysis by using EDX-9100, the adult leaves of approximately the same age were taken from fully expanded sun-exposed ones and cut into small blocks with 0.5 cm^2 along the vein. The material for EDX observation was macerated in FAA solution about 48 h, and dehydrated in a series of gradient alcohol. The plant material was then mounted on stubs with double-sided adhesive tape and the specimens were examined and photographed at 30 kV under a Quanta 200 scanning electron microscope at room temperature in order to obtain the weight and atom percent of elements (WT% and AT%) (including C, O, Mg, P, Ca and Mn) in the leaf cells of two different species of *Flos Lonicerae*.

The Nongla Karst Experimental Site is located in Guling town, Mashan county, Guangxi, China, a typical karst Fengcong (depression) mountainous area (108°19' E, 23°29' N). Temperature ranges from 8 to 30°C, with the annual average of 19.84°C (Li et al. 2007). Annual precipitation is about 1750 mm and annual evaporation is about 1278 mm. At this area, the main lithology is a gently dipping, thick, marl-silica dolomite of the Donggangling Formation, which is middle Devonian, resulting in rainfall flow into the epikarst zone along the cranny, followed by the soil drought (Zhang et al. 2005). As a result, the Nongla Karst Experimental Site has the common characters of karst environment, i.e. soluble rock, soil scarcity, calcium-rich, alkaline and water leakage.

Soil material. The soil profile at the area shows three main layers: (1) above the rock of a gently dipping, thick, marl-silica dolomite of the Donggangling, from 50 to 40 cm depth (B soil horizon); (2) from 40 to 20 cm depth, a brown-yellow clayey layer, with an aggregated structure (AB soil horizon); (3) from 20 to 0 cm depth, a humus layer (A soil horizon).

The soil samples were collected near the rhizosphere soil around the two species of *Flos Lonicerae* on 20 April 2005 with drills at different sites along the soil profile. Soil samples from different layers were mixed for the laboratory analysis.

The soil samples were dried to constant weight at 40°C and then homogenized and analyzed by the modified Walkley-Black procedure (dichromate oxidation with external heating) (Allison 1965). The total P concentration in the soil was analyzed at the 700 nm by spectrophotometry of ammonium molybdate. The total amount of K in soil was analyzed by acid-leaching atomic absorption spectrometry. The total contents of Ca and Mg in soil were titrated by ethylene diamine acid (EDTA). The total contents of B, SiO_2, Mn, Cu, Zn, Fe and Na were checked by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, Optima 3000 DV Perkin-Elmer) after digestion of 1 g biomass in concentrated HNO_3. The soil available concentrations of B, P, K, SiO_2, Ca, Mg, Mn, Cu, Zn, Fe and Na were extracted with AB-DTPA method (Jones 1990) and analyzed by ICP-MS.

Statistical analysis. The statistical analysis of the data was made by the mean values ± SE. Significance was determined by Student's *t*-test for unequal variance or by completely randomized design followed by a least-significant-difference test (Milton and Tsokos 1983).

RESULTS AND DISCUSSION

Soil characters of the samples

Table 1 shows the total amount of eleven elements in the soil at the Nongla Karst Experimental

Site where *Lonicera japonica* Thunb. and *Lonicera confuse* grow. The eleven elements in the soil of A and B soil horizon reveals the same pattern: SiO_2 > Fe > Ca > Mg > K > Na > Mn > P > Zn > B > Cu. In the AB soil horizon, the elements have the similar sequence except Mn and Na (Mn > Na).

In addition, the efficient value (%) and the soil available concentrations in the A soil horizon are presented as: Ca > Mg > Cu > Mn > K > Na > P > B > SiO_2 > Zn > F, the efficient value (%) and the soil available concentrations in the AB soil horizon are presented as: Ca > Mg > Cu > Mn > Na > K > P = SiO_2 > B > Zn > Fe, and the efficient value (%) and the soil available concentrations in the B soil horizon are presented as: Ca > Mg > Cu > Mn > Na > K > P > B > SiO_2 > Zn > Fe.

Main element characters in the plant cell

As presented in Figures 1 and 2 and Tables 2 and 3, the element contents in the cell of *Lonicera japonica* Thunb. and *Lonicera confuse* are different, reflecting their chemical composition and biogeochemical characteristics caused by the karst environment. *Lonicera japonica* Thunb. and *Lonicera confuse* have the similar WT% in the cell. Except C and O, Ca is the main element in their cell and its content has the negative correlation with Mg ($r = -0.156$, $P < 0.01$). The content of P is inferior to Ca ($r = 0.868$, $P < 0.01$).

From the WT% results, *Lonicera japonica* Thunb. shows the higher content of Mg, P and Mn and is 1.4, 2.5 and 3.5 times higher than *Lonicera confuse*, with the exception of Ca. Due to the low content of Fe in the *Lonicera confuse*, which was not checked out by EDX-9100, the Fe content in the cell of *Lonicera japonica* Thunb. is quite high.

In addition, the AT% in the leaf cells of two species of *Flos Lonicerae* shows the same sequence with WT% (Figures 1 and 2; Tables 2 and 3), probably caused by the selective absorbency.

The plant needs to absorb the elements from the soil horizon in the geosphere in order to get grown. The element concentration is affected by the nutrition loss and formation equation of the elements in the soil cycle, resulting in the limit of the nutritional elements supplemented to the plants (Du and Zhong 1998).

As shown in Table 1, the total amount of soil elements provides the primary issue on soil background of the Nongla Karst Experimental Site. The hydrochemical type of soil is therefore HCO_3-Ca-Mg, which reflects the control of the argillaceous and

Table 1. Total and liable content of elements in soil in Nongla, Guangxi

Horizon name	Contents of elements	B	P	K	SiO_2	Ca	Mg	Mn	Cu	Zn	Fe	Na
A	total content	106.50	723	6367	343900	32631	19227	2006.50	23.15	431.60	131300	2018
AB	total content	87	602	5524	343200	25237	17844	1780	18.91	411	130000	1048
B	total content	76.30	571	5986	338900	23268	16773	1367	23.13	414.80	132500	1437
	average	89.93	632	5959	342000	27045.33	17948	1717.83	21.73	419.13	131266	1501
	CV(%)	17.03	12.71	7.08	0.79	18.25	6.85	18.88	11.24	2.62	0.95	32.52
A	liable content	0.32	2.36	39.65	684.50	2568.50	990.60	23.62	0.45	0.50	13.98	10.40
AB	liable content	0.16	1.62	28.50	925.90	2039.90	838.50	17.51	0.23	0.19	15.21	9.38
B	liable content	0.15	2.29	29.27	384.50	1650.60	741.43	12.89	0.32	0.21	14.39	9.65
	average	0.21	2.09	32.50	664.97	2086.33	856.84	18.01	0.33	0.30	14.53	9.81
	CV(%)	45.42	19.55	19.18	40.79	22.08	14.66	29.89	33.18	57.83	4.31	5.39
A	efficient value (%)	0.30	0.33	0.62	0.20	7.87	5.15	1.18	1.94	0.12	0.01	0.52
AB	efficient value (%)	0.18	0.27	0.52	0.27	8.08	4.70	0.98	1.22	0.05	0.01	0.90
B	efficient value (%)	0.20	0.40	0.49	0.11	7.09	4.42	0.94	1.38	0.05	0.01	0.67

The unitage of total and liable content of soil elements is mg/kg; *CV* – coefficient of variation

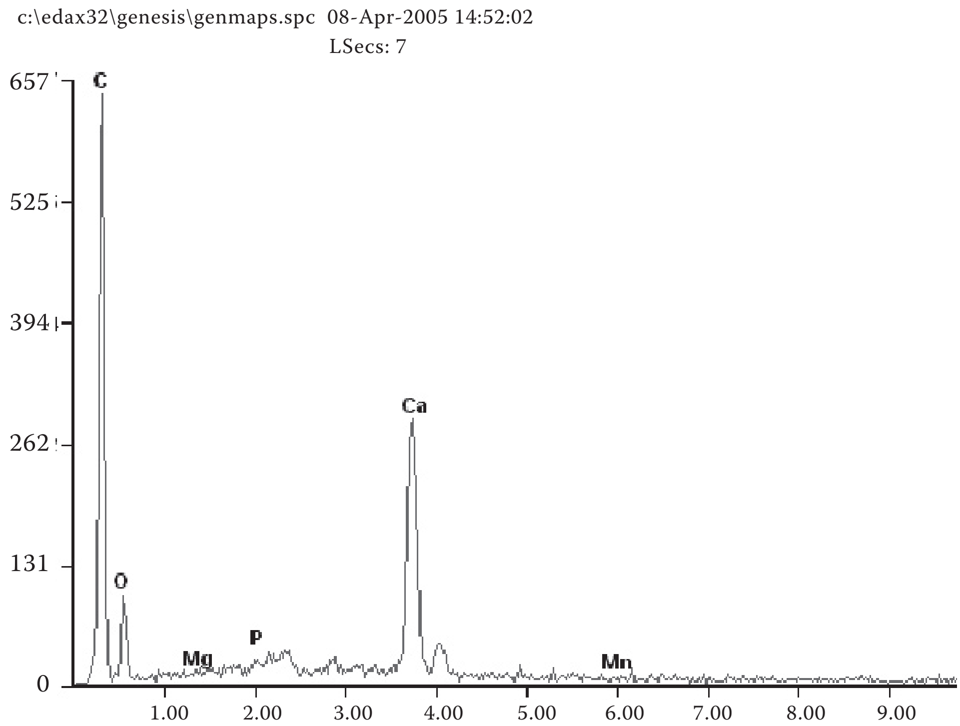

Figure 1. The content of elements in the leaf cell of *Lonicera confuse*

siliceous dolomite of the Donggangling Formation of Devonian at the site.

However, the nutritional elements can be assimilated and transferred into the plants or not mainly depending on the migration velocity of soil elements and its available concentrations, even though the total amount of the elements in the soil plays an important role (Wang and Cheng 2004). Then, we need to use the soil element efficient value (%) and the soil available concentrations to evaluate the nutritional element condition that can be used by the plant. From the results of the

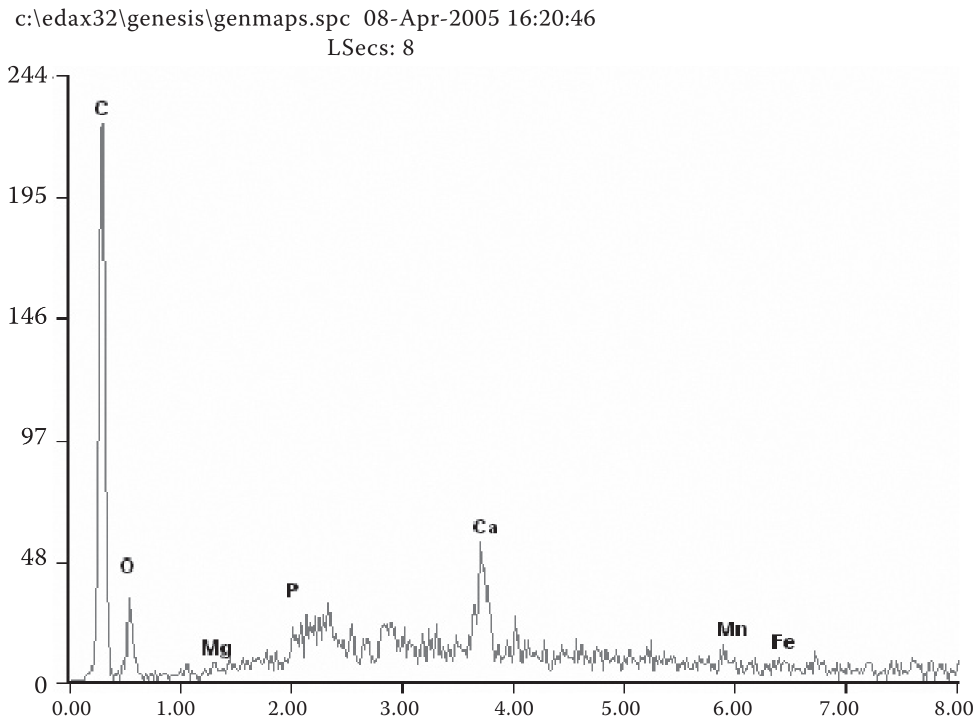

Figure 2. The content of elements in the leaf cell of *Lonicera japonica* Thunb.

Table 2. The content of elements in the leaf cell of *Lonicera confuse*

Element	WT%	AT%
C	70.19	80.53
O	17.75	15.29
Mg	0.09	0.05
P	0.30	0.14
Ca	11.43	3.93
Mn	0.24	0.06

WT – weight percent of element; AT – atom percent of element

Table 3. The content of elements in the leaf cell of *Lonicera japonica* Thunb.

Element	WT%	AT%
C	75.51	83.51
O	16.89	14.03
Mg	0.13	0.07
P	0.81	0.35
Ca	4.87	1.61
Mn	0.85	0.21
Fe	0.95	0.23

WT – weight percent of element; AT – atom percent of element

soil element efficient value (%) and the soil available concentrations, it is shown that the efficient value (%) and the soil available concentrations in the soil have the same pattern of Ca > Mg > Cu > Mn in the different horizons, and the micronutrients are quite low reflecting the characteristics of the karst soil at Nongla, including calcium-rich, alkaline, soil scarcity (Yuan 2001). Moreover, the coefficient of variation (%) can also reflect the migration velocity of nutritional elements at this site. In Table 1, Ca, Mn and P have the high coefficient of variation (%) and total concentrations, indicating that the three elements in the soil have the ionic condition and can be effectively absorbed by plants. Cu and B cannot be effectively absorbed by plants because of their low liable contents, even though their coefficient of variation (%) and total concentrations are quite high. Si has the high coefficient of variation (%), total concentration and liable content, but it cannot be assimilated by plants as the result of its SiO_2 condition in the soil. From these results, it can be seen that the soil at Nongla has the low nutrient content due to its karst geology background.

According to floristic theory, the soil, climate and human activity can affect the element concentrations in the leaf cells of plants, but the plants have the special features of selective absorbency to elements, which controls the element contents in the plant cell (Du and Zhong 1998, Wang and Cheng 2004). By analyzing the relationship of Ca, Mg and P, the content of Ca is shown higher while Mg is at the lowest in the cell of two different species of *Flos Lonicerae* (r = –0.156, P < 0.05) and the content of P is inferior to Ca (r = 0.868, P < 0.01). Moreover, the research on the relationship of soil and the plants has shown that this result is perhaps caused by the characteristics of local biogeochemistry. Ca is mainly absorbed by plant with the help of transpiration and is accumulated in the cell, becoming the predominant element in the cell. The accumulation of Ca in the plant cell will affect the absorbency and the content of other elements in the cell of *Flos Lonicerae*. As a result, the content of other elements in the *Flos Lonicerae* are different through synergistic action and antagonistic action, affecting its quality and officinal value. This was also noticed by Zhang et al. (2001) in his study on the relationship between element content in soil and tobacco. From the above results, it can be observed when the plants absorb the elements from soil; plants have the synergistic action between Ca and Mg and antagonistic action between Ca and P, which will affect other element concentration. As shown in Figures 1 and 2 and Tables 2 and 3, the AT% in the cell can also reflect the element concentrations and condition, it has the same pattern with WT%, perhaps caused by the selective absorbency in the leaf cells of two species of *Flos Lonicerae*.

In order to study the biogeochemical effect of soil on the bioactive components in the two species of *Flos Lonicerae*, the authors also discussed their quality under the biogeochemical effect. The bioactive components of the dried buds from *Lonicera japonica* Thunb. and *Lonicera confuse* are widely used as the traditional Chinese medicine (TCM) herb; they are usually applied to estimate the quality of these two species. Because *Lonicera japonica* Thunb. is introduced from North China to South China, the physical conditions such as light and temperature at the Nongla Karst Experimental Site are different from its original environment. The different daylight hours, as the limit factor to the land life, can affect their photosynthesis and florescence. In South China, the Nongla Karst Experimental Site belongs to short light area and *Lonicera japonica* Thunb. does not flower, as there is no enough light to activate the florescence of *Lonicera japonica* Thunb. Though, *Lonicera japonica* Thunb. can grow up very quickly to cover the bare karst area, reduce water and soil loss, and provide a very effective way to rebuild the biogeocenose and furthermore, the rock desertification at karst area can restrict the popularization of *Lonicera japonica* Thunb. because it has low economic value which cannot help the local people to get rid of poverty at karst area in Southwestern China (Li 2000, Floh and Handro 2001).

The quality of *Flos Lonicerae* (Jinyinhua in Chinese) widely used as traditional Chinese medicine (TCM) herb, is mainly affected by soil environment. It is because some special microelements control the quality of the herbs and have the chelation with the sickness when is dosed by the patient, according to the theory of traditional Chinese medicine (Guo et al. 2002, Yang 2003). The GAP base was formed in China to plant particularly Chinese herbs. Under the affection of soil environment, the herb can absorb and get rich in the elements from the soil, which reflects the environment affection. *Lonicera confuse*, as the original species at karst area, has been adapted to this environment and has the calcium/magnesium-rich character, with low microelement concentrations. When the transpiration takes place, Ca can be absorbed into the plant and get rich in the young part of the body (Du and Yang 1999). Moreover, the content of Ca can also affect the content of other elements through synergistic and antagonistic action. And, the element contents in the plant also are controlled by its germplasm. The Ca content of *Lonicera confuse* is therefore higher than *Lonicera japonica* Thunb., resulting in the low content of Mg, P, Mn and Fe. Due to the low content of the microelement, the dried buds of *Lonicera japonica* Thunb. and *Lonicera confuse* at this site cannot reach the GAP standard of *Flos Lonicerae* (Jinyinhua in Chinese). If the local governments desire to bring the rock desertification under control and promote the local economy, they should look for other plants or apply plant breeding and genetics methods to replant *Lonicera japonica* Thunb. and *Lonicera confuse.*

The biogeochemical characteristics of the plants at karst area are controlled by its special environment and can reflect the influence of the relationship between the soil and the main element in the plant cell. It is because the content of elements in the *Flos Lonicerae* are determined by synergistic action antagonistic action. The physical condition can affect its element contents in the plant cell and its quality, resulting in the difference of the quality and officinal value of the *Flos Lonicerae*.

Moreover, through EDX in the EMS, it is easy and simple to test the element contents in the living plant and the herbs quality. Based on the important information of the environment influences from the primary search, it is easy to introduce some important and economic plants to control the rock desertification at karst area and help the local people with better economy in Southwestern China. By applying the EDX data, more and more special characters of plant-soil ecosystem can be discovered in the future.

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