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RELATIONSHIPS BETWEEN THE SELENIUM CONTENT IN FLUE-CURED TOBACCO LEAVES AND THE SELENIUM CONTENT IN SOIL IN ENSHI, CHINA TOBACCO-GROWING AREA

ZICHENG XU*, HUIFANG SHAO, SU LI AND CONG ZHENG

College of Tobacco Science, Henan Agricultural University, 450002 Zhengzhou,China
**Corresponding author's e-mail: zichengxu@126.com*

Abstract

Relationships between the selenium content in flue-cured tobacco leaves and the selenium content in soil in Enshi tobacco-growing area were analyzed. The results indicated that: (1) The selenium content in flue-cured tobacco leaves in Enshi was high, ranging from 0.30 to 29.30 mg·kg^{-1} with the mean of (8.37±5.83) mg·kg^{-1}.The content of selenium in flue-cured tobacco among different positions was X2F>C3F>B2F, among different altitudes was medium altitude(800m~1200m)>high altitude(≥1200m)>low altitude (<800m). (2) The selenium content in soil in Enshi tobacco-growing area was high，ranging from 0.28 to 7.03 mg kg^{-1} with the mean of (2.56±1.64) mg kg^{-1} . Most of the soil was selenium-rich and high-selenium soil, and 34.44% of soil was excessive-selenium soil. As the soil thickness deepened, the selenium contents in soil became lower and lower, and the same trend was observed in the selenium content of flue-cured tobacco leaves at different altitudes. (3) There was significantly positive-correlation between the selenium content in soil and the selenium content of flue-cured tobacco leaves (correlation coefficient was 0.949). Based on the correlation analysis, the regression equation, $\hat{y} = -0.1082x^3 + 1.2494x^2 - 1.9628x + 8.1647$ was established between soil available selenium and the selenium content in flue-cured tobacco leaves for 72 samples. Regression equation reached a significant level by F-test.

Introduction

Since selenium was identified as one of the most necessary microelement of human body by WHO in 1973, the research on selenium has developed rapidly (Awasthi *et al*., 1975). Early interest in Se was related particular to its toxic effects, but later investigations showed that insufficient Se supply can result in several disorders, such as muscle degeneration and physical damage to cell membrane (Khan & Ashraf, 2008)**.** The references released by WHO indicated that more than 40 countries belonged to low-selenium and selenium-poor regions all over the world (Beath, 1964). Our survey showed that 72% of counties in China are geared to low-selenium and selenium-poor areas and part of regions from Heilongjiang, Inner Mongolia, Gansu, Qinghai to Sichuan are so poor of selenium, that Keshan diseases, Kaschin-beck disease and other diseases of selenium-poor occur in the regions (Wu *et al*., 1997). It has been reported that low-selenium in food and water can easily cause cancer, cardiovascular disease, arthritism and other diseases (Yates *et al*., 1986). Increasing the selenium content of tobacco leaves not only descends down toxicity of tar in cigarette but improves blood selenium water level of smokers. (Xu *et al*., 2011). Low selenium content far below critical level in soil was not suitable for plant growth. According to these results, soil selenium concentrations showed sampling period effects. Ahmad *et al*., (2009), Bodgen (1975) and Shao & Xu (2011) analyzed the selenium content in tobacco found that the selenium content in Mexico and Columbia with low incidence of lung cancer was three times as much as that in England and America with high incidence of lung cancer. In consideration of the soil-plant-animal and human ecosystem, soil is the most fundamental factor. Therefore, researches in many countries on the selenium content in soil and plant have been paid much attention for a long time. Enshi has been considered as one of the most important tobacco-producing areas in Hubei province and the only high-selenium content area in the world by far, which makes few systematic reports on selenium content in soil and flue-cured tobacco but mainly concentrates on tobacco physiology and the selenium accumulation of flue-cured tobacco (Guo, 1998; Yang, 2000; Gao, 2006; Pan, 2006). In order to further improve the intrinsic quality of tobacco and develop the selenium content of tobacco in Enshi, this paper is focused on the basis of the soil and flue-cured tobacco in Xuan'en and Xianfeng and distribution of selenium content in different soil layer at different altitudes and different flue-cured tobacco positions in a systematic way and to provide scientific basis for producing the characteristic raw materials with suitable selenium content.

Materials and Methods

Sample collection

Soil samples: Ninety tobacco planting soil samples were collected at different altitudes from 0~20cm, 20~40cm and 40~60cm depth through the application of GPS orientation technology, to the study on selenium content in soil at Xuan'en and Xianfeng in 2006.

Collection of tobacco leaves after flue-curing: According to the different grades and altitudes, we selected 72 flue-cured tobacco samples in Xuan'en and Xianfeng in 2006. The altitude of each sample section was recorded with the help of GPS concluding Yunyan87, Yunyan85, K326 etc. and the grades of each sample section concluding X2F, C3F and B2F. Sample grades

were rated by the special full-time staff in accordance with the standard of "GB 2635-92 flue-cured tobacco" and the qualification rate was 100%. Each sample was weighed 3.0kg applied to determine the chemical composition. The tobacco samples were filtered by 60 mesh sieve after oven drying and smashing into pieces.

Index determination methods

The analysis of selenium content in soil and of flue-cured tobacco was determined according to the reference materials (Pan *et al*., 2006).

Statistics analysis

The data was processed and analyzed by using SPSS17.0 software.

Results and Measurements

The selenium content in flue-cured tobacco leaves in Enshi was high, ranging from 0.30 ~ 29.30 mg/kg with the mean of (8.37±5.83) mg/kg. The result of the tobacco selenium content among different grades was X2F>C3F>B2F (Table 1). But there was no significant difference among different positions. Additionally, since the variation coefficient (VC) of the flue-cured tobacco selenium content among each position was high, the soil environment, climate and breeds have a great effect on flue-cured tobacco selenium content with the rate of 71.46% in central leaves. The result showed that the effective utilization of soil selenium content had to be settled urgently for the instability of tobacco selenium content in this area while it belonged to the high-selenium area. The scope of the Kurtosis coefficient was high, which was greater than 0 among different grades of tobacco and the data mostly embodied a concentrated reflection of the mean. As can be seen from Table 2, the main performance of selenium of flue-cured tobacco at different elevations was middle altitude (800 ~ 1200m) > high altitude (≥ 1200m) > low altitude (< 800m). The difference of selenium content among middle altitude, high altitude and low altitude reached a significant level.

Table **1. Comparison of selenium content of flue-cured tobacco of different gradings in Enshi, China tobacco-growing areas.**

Grading	Samples	Mean ± Std.	Range	VC (%)	Skewness	Kurtosis
B2F	24	7.78 ± 5.51 a	1.93~23.60	70.82	1.15	1.21
C3F	24	8.27 ± 5.91 a	0.30~29.30	71.46	6.79	2.71
X2F	24	9.06 ± 6.22 a	0.30~23.40	68.64	0.34	1.09

Table **2. Comparison of selenium content of flue-cured tobacco in different altitudes in Enshi, China tobacco-growing areas.**

Altitude (m)	Samples	Mean ± Std.	Range	VC (%)	Skewness	Kurtosis
< 800	24	5.76 ± 3.49 b	1.60~17.40	60.52	4.24	1.57
800~1200	24	10.13 ± 7.77 a	0.30~29.30	76.67	0.15	1.01
≥1200	24	9.21 ± 4.61 a	3.90~23.40	50.08	2.24	1.75

Note: Different letters in the table indicated the significant differences at the 0.05 level, the same next.

Analysis of soil selenium content in Enshi

The total condition of soil selenium content: The distribution of soil selenium content in China is not well-distributed. Based on the study of Keshan disease and poor-selenium environment, Tan (1996) divided the limitation of selenium content in soil.

The soil in which selenium content was smaller than 0.125mg/kg belonged to selenium deficient oil. The soil with the selenium content of (0.125~0.175mg/kg) belonged to soil containing a little selenium. The soil with the selenium content of (0.175~0.450mg/kg) belonged to soil containing adequate selenium. The soil with the selenium content of (0.450~2.000mg/kg) belonged to soil containing full selenium. The soil with the selenium content of (2.00~3.00mg/kg) belonged to soil containing large selenium. The soil with the selenium content of (≥ 3.000 mg/kg) belonged to soil containing excess selenium. As shown in Table 1, we can concluded that most of the soil in Enshi belonged to soil containing full selenium or large selenium, 34% of which belonged to soil containing excess selenium, up to 7.03mg/kg (Table 3).

Table **3. Frequency distribution of selenium content in soil in Enshi, China tobacco-growing area.**

Selenium content ($mg.kg^{-1}$)		Samples	Proportion (%)
Selenium deficiency soil	< 0.125	0	0.00
Soil contain a little selenium	0.125~0.175	0	0.00
Soil contain adequate selenium	0.175~0.450	4	4.44
Soil contain full selenium	0.450~2.000	32	35.56
Soil contain large selenium	2.000~3.000	23	25.56
Soil contain excess selenium	≥3.000	31	34.44

As shown in Table 4, the average content of soil selenium in Enshi was nearly 5 times higher than that in Guizhou and seven times higher than that in Mainland. The average content of soil selenium in Xuan'en reached 3.01 mg/kg, which was four times higher than in Hongkong, one of the selenium-rich soil in China, with its larger variation coefficient reaching 57.64%. This indicated that the selenium contents in tobacco plantings soils were much different among different locations and influenced deeply by soil environment factors.

Table **4. Comparison of selenium content between Enshi and Guizhou, Hong Kong and inland of China (mg.kg-1).**

Area	Mean	Median	Minimum	Maximum	VC (%)
Xuan'en	3.01	2.88	0.38	7.03	57.64
Xianfeng	2.11	1.82	0.28	6.32	66.88
Guizhou	0.37	—	0.06	1.33	59.61
Hong Kong, China	0.76	0.74	0.07	2.26	54.82
Inland of China	0.29	0.21	0.05	0.99	87.97

Different distribution character of selenium content in different soil depth: The result of analysis of the soil selenium content in different tobacco planting areas in Enshi was listed in Table 5. As shown in Table 5, the selenium content in the soil of 0~20 cm depth was highest, reaching (3.73 ± 1.89) mg/kg. The difference between 20~40cm and 40~60cm soil layer reached a significant level. The soil selenium content became lower as the soil thickness became deeper. The variation coefficients of total selenium content in different layer soil were large and the ones in deeper soil were larger than in surface soil. From the kurtosis and skewness, kurtosis coefficients of selenium content in different arable layer soils were greater than 0, considered as leptokurtic and the data was mostly concentrated near the mean. Skewness coefficients of selenium content in different arable layer soil were greater than 0, considered as the positive skewed peak.

Table **5. Status of selenium content in different soil depths in Enshi tobacco-growing areas.**

Depth (cm)	Samples	Mean ± Std.	Range	VC (%)	Skewness	Kurtosis
0-20	30	3.73 ± 1.89 a	0.74~7.03	50.85	2.78	1.56
20-40	30	2.04 ± 1.11 b	0.28~3.86	57.68	0.31	0.62
40-60	30	1.92 ± 1.14 b	0.21~4.74	55.86	0.25	0.65

Different distribution character of selenium content in different altitudes: The result of analysis of the selenium content in tobacco planting soil of different altitudes in Enshi region was listed in Table 6. As shown in Table 6, selenium content at middle altitude (800~1200m) in tobacco plantings soil was highest with the mean of (3.17 ± 1.79) mg/kg. The performance of the soil selenium at different altitudes above sea level was middle altitude (800~1200m) > high altitude (≥ 1200 m) > low altitude (< 800 m), which was the same trend as the selenium content of flue-cured tobacco at different altitudes. The correlation analysis of the selenium content of flue-cured tobacco and the selenium content in tobacco planting soil at different altitudes were compared and the correlation coefficient reached 0.868 ($p < 0.01$). The difference of the selenium content in tobacco planting soil between middle to high elevation and low elevation reached significant at the level of 0.05. The variation coefficient of selenium content in tobacco planting soil at different altitudes were large, which indicated that the content at different locations varied greatly although the selenium content in Enshi was very high. As was seen from the kurtosis and skewness, the kurtosis coefficients of selenium content in tobacco planting soil at low altitude and high altitude were greater than 0, considered as leptokurtic. The data was mostly concentrated in the vicinity of mean. The kurtosis of selenium content in the tobacco planting soil at middle elevation was less than 0, considered as the width of the peak level and the data scattered. The skewness of selenium content in tobacco planting soil at different altitudes was greater than 0, considered as the positive skewness.

Table **6. Comparison of selenium content in soil at different altitudes in Enshi, China tobacco-growing areas.**

Altitude (m)	Samples	Mean ± Std.	Range	VC (%)	Skewness	Kurtosis
< 800	30	1.84 ± 1.19 b	0.21~5.61	64.73	1.73	1.08
800~1200	30	3.17 ± 1.79 a	0.53~6.87	56.58	-0.19	0.54
≥1200	30	2.67 ± 1.63 a	0.28~7.03	61.03	0.97	0.87

The analysis of the relationship between the total selenium content in soil and the selenium content of the flue-cured tobacco

The trends of total selenium content in soil and selenium content of tobacco: Soil selenium content was divided into 6 groups according to Se content < 1.00, 1.00 ~ 2.00, 2.00 ~ 3.00, 3.00 ~ 4.00, 4.00 ~ 5.00 and ≥ 5.00 mg/kg. The total selenium content in soil and the selenium content of flue-cured tobacco were calculated in each group. The relationship between the total selenium content in soil and the selenium content of flue-cured tobacco was analyzed (Table 1). The result of correlation analysis showed that there was a significant positive correlation between them with the positive correlation coefficient being 0.949. As shown in Tab. 1, when the total selenium content in soil was low (< 2.00 mg/kg), accompanying with the selenium content in soil increased slowly, the selenium content of flue-cured tobacco increased quickly. When the total selenium content in soil was high (2.00~5.00mg/kg), accompanying with the selenium content in soil increased quickly, the selenium content of flue-cured tobacco increased quickly. When the total selenium content in soil was above a certain value, the varying speed of selenium content of flue-cured tobacco became slow.

One-way ANOVA was applied to compare the difference of selenium content of flue-cured tobacco in different groups which were divided by the total selenium content in soil, and the test showed a significant level. The multiple comparisons were made on this and the result was shown in Table 7. It showed that the difference of the selenium content of flue-cured tobacco among groups reached a significant level (P< 0.01). The tobacco selenium content showed no significant difference among different groups when the total selenium content in soil was under 7.01 mg/kg. The tobacco selenium content was showed great significant difference among different groups when the total selenium content in soil was beyond 2.01~3.00 mg/kg. When the total selenium content in soil was beyond 2.01~3.00mg/kg, the selenium content of tobacco became higher accompanying with the total selenium content in soil increased (Fig. 1).

Table **7. Effect of total selenium content in soil to selenium content in flue-cured tobacco leaf.**

Total selenium content in soil (mg·kg^{-1})	Samples	Selenium content in tobacco (mg·kg^{-1})
< 1.00	16	7.84 D
1.01~2.00	13	8.40 D
2.01~3.00	18	8.54 D
3.01~4.00	12	11.87 C
4.01~5.00	3	15.97 B
≥5.00	10	17.57 A

Note: Different letters in the table indicated the significant differences at the 0.01 level

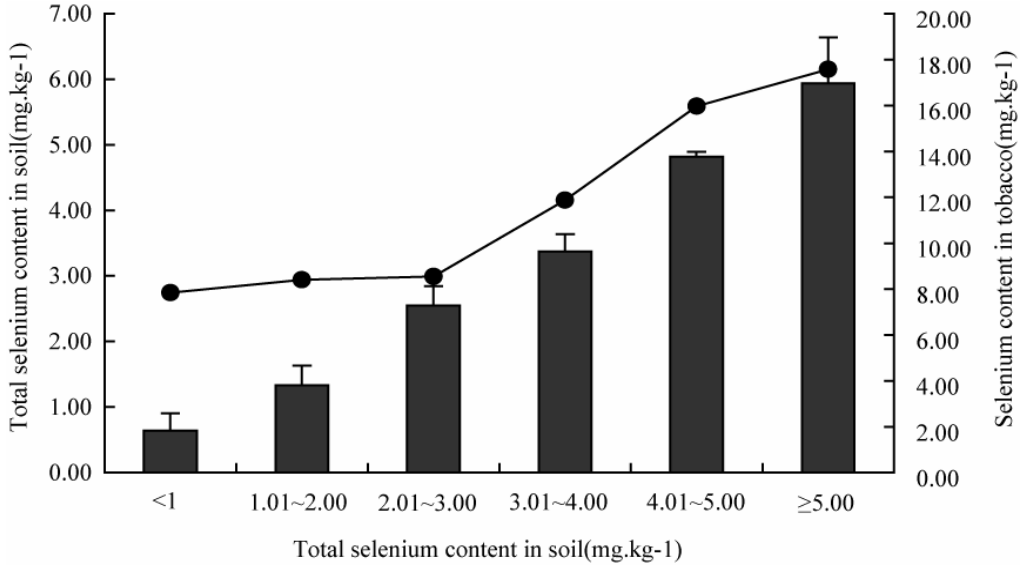

Fig. 1. Change of total selenium content in soil and selenium content in flue-cured tobacco leaf.

The regression analysis of the total soil selenium content on tobacco selenium content: Considering 0.5mg/kg of the total soil selenium content as the class interval, we divided the soil samples into 14 groups and counted up the total selenium content of each group and the selenium content of the corresponding flue-cured tobacco samples. Then the regression relationship between the total selenium content in soil and the selenium content of flue-cured tobacco was analyzed in the Tab. 2. The relationship between them was significant and the regression equation was derived as $\hat{y} = -0.1082x^3 + 1.2494x^2 - 1.9628x + 8.1647$. The F tests indicated that the regression equation reached a significant level of 0.01. When the soil selenium content was in the range of 2.00~6.00mg/kg, accompanying with the total selenium content in soil improved, the selenium content of flue-cured tobacco grew quickly (Fig. 2).

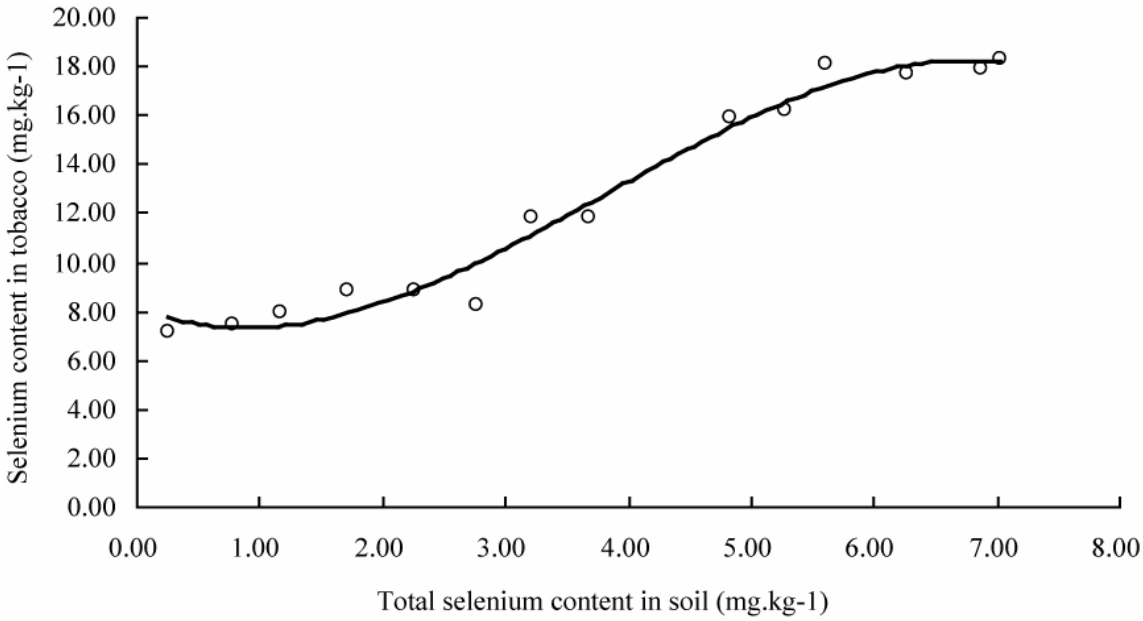

Fig. 2. Relationship of soil selenium and selenium content in flue-cured tobacco leaf.

Conclusion and Discussion

(1) Some researchers have shown that improving the selenium content in cigarette is beneficial to reduce the main diseases caused by smoking (Bodon, 1981). Since the smoking and health issues become more and more prominent, pollution-free production of flue-cured tobacco was an important way to improve the security of tobacco (Yuan *et al*., 1994, Ma *et al*., 2001). Studying on the selenium content in tobacco has become one of the most important issues in cigarette production field in China. Enshi is one of the important tobacco-producing areas in Hubei province and the high-selenium areas in the world. It has an important effect on characteristic tobacco in this region to study on the characteristic of flue-cured tobacco systematically, and it is of benefit to creating the brand of them own and offering scientific basis for Chinese cigarette material with suitable selenium content (Xu *et al*., 2011). The mean of the selenium content of flue-cured tobacco in Enshi was (8.37 ± 5.83) mg/kg, ranging from 0.30 to 29.30mg/kg. The main performance of selenium content was X2F > C3F > B2F, but the difference of each grade was not significant. The variation coefficient of selenium content of flue-cured tobacco at each part was larger which indicated that the selenium content was not only affected by the planting soil, climate and other factors, but also not stable while this region was belonged to high-selenium areas. The efficient use of selenium in soil was still a serious problem. The main performance of the selenium content in tobacco among different altitudes was middle altitude (800~1200m) > high altitude (≥ 1200m) > low altitude (< 800m). The difference of middle altitude or high altitude and low altitude reached a significant level.

(2) Tan (1996) worked on Keshan disease and low selenium environment in China and divided the limits of ecological value about selenium content. According to the classification, the total soil in Enshi belonged to rich-selenium soil. The 34.44% of the total soil was belonged to excess-selenium soil up to 7.30 mg/kg. As it can be seen from Table 4, the average of soil selenium content in Enshi is nearly 5 times higher than that in Guizhou (Zhang *et al*., 2005) and 7 times higher than that in Mainland. The average soil selenium content in Xuan'en reached 3.01 mg/kg, which was 4 times higher than that in Hongkong, a rich-selenium area in China, with its larger variation coefficient reaching 57.64%. This indicated that the selenium content in tobacco planting soils was much difference among different locations and influenced deeply by soil environment factors.

With the thickness of soil layer became deeper, the total selenium content in soil of different soil layers in Enshi became lower. However, the variation coefficient of selenium content in different soil was large and the variability in underlying soil was higher than surface soil. It was caused by so long term cultivation in surface soil that the selenium content has been close to the average, while the underlying soil has been little affected by cultivation relatively which was controlled by soil sorts (Yang *et al*., 1998). The selenium content in soil of different altitudes in Enshi regions was that the content of middle altitude was higher than others, and the reason was that the soil at the altitude (900~1000m) was rich-selenium carbonaceous rocks (carbonaceous chert, carbonaceous siliceous rocks and carbonaceous shale, the local residents considered these rocks as "stone coal"), so the soil produced by the rocks oxidized and weathered was full of selenium (Zhu & Zhang , 2005).

(3) Based on soil-tobacco system, Xu *et al*., (2007, 2008) and Wang & Xu (2007) had done thorough researches and analysis of the relationship between nutrients in soil and mineral elements in flue-cured tobacco. In this study, the selenium content in soil in Enshi region and the total selenium content in flue-cured tobacco

was analyzed in a similar method. The result showed that when the total selenium content in soil was low, accompanying with the total selenium content in soil increased, the selenium content of flue-cured tobacco increased slowly. When the total selenium content in soil was high, accompanying with the total selenium content in soil increased, the selenium content of flue-cured tobacco increased quickly. Variance analysis methods were applied to compare the difference of the selenium content of flue-cured tobacco among groups which was divided by the difference of the total selenium content in soils, and the results indicated that the difference of the selenium content of flue-cured tobacco among groups reached a significant level ($P< 0.01$). Regression analysis methods were used to analysis the relationship between the total selenium content in soil and the selenium content of flue-cured tobacco and the same conclusion was made finally.

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