The geographical distribution of soil elements determines the boundaries of the Daodi-zone of medicinal plant (Ligusticum chuanxiong Hort.)

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Abstract. Understanding the boundary of plants' best production zone is of great significance to the rational introduction cultivation. We set up 9 sample points in the center-zone and 28 sample points extending eastward along the longitude. Then, we were collecting the bulk soil of L.chuanxiong, testing soil properties and elements. Later, a multi-point cultivation test was carried out on L.chuanxiong, the

25   content of elements in the plant measured by ICP-MS, and HPLC detected the active ingredients. Based on hierarchical clustering and PCA analysis, the study zone's soil element was divided into three groups. In the L.chuanxiong cultivation experiments, we found Ligustilide in rhizomes was positively related to Se and Sr and negatively related to Mn and the chemical elements. This study successfully verified that the medicinal plant's Daodi-zone has a natural boundary, and L.chuanxiong's Daodi-zone may be from

30   east of Dujiangyan to the west edge of Longquan, rich in Na, Mg, K, and Low Mn is the soil characteristics of the area. Moreover, we put forward a method to identify L.chuanxiong's Daodi-zone

based on characteristic soil elements, which can also provide a practical basis for dividing other economic plants' growth environment.

35   **Key words:** Soil elements;Daodi-zone;geo-boundary; *Ligusticum chuanxiong* Hort.

1 INTRODUCTION

Soil is the environmental basis of plant growth, a variety of elements in the soil directly involved in plant growth and development, morphological construction and secondary metabolism, and then affect the accumulation of some compounds in plants, such as trace element boron (B) deficiency will lead to

40   changes in cell wall structure(Guerriero et al., 2018;Matthes et al., 2020); B deficiency in tobacco leaves will induce the accumulation of soluble sugar and phenolic compounds (chlorogenic acid and coffee polyamine conjugates)(Camacho-Cristóbal et al., 2004). Besides, heavy metal element stress increases the accumulation of secondary metabolites by stimulating the plant to produce an immune response (Lajayer et al., 2017b). Since the soil properties are influenced by soil mass, topography, and

45   geochemistry, it has a significant heterogeneity in geographical distribution, directly or indirectly affecting the absorption of plant elements and secondary metabolic synthesis (Guerriero et al., 2018;Bonari et al., 2019). At present, many studies have reported significant regional Heterogeneity in the accumulation of active ingredients in plants (Yang et al., 2018a;Li et al., 2020). In Sinopodophyllum hexandrum (Royle) T.S. Ying (Liu et al., 2015), conditions in Jingyuan, Ningxia Province, and Yongdeng,

50   Gansu Province, are suitable for the production of podophyllotoxin and lignans, whereas conditions in Shangri-La, Yunnan Province, and Nyingchi, Tibet, are suitable for the production of quercetin and kaempferol. Simultaneously, Catechin and free amino acids in Green tea (Wen et al., 2020) are also affected by the cultivation location. Zhang et al. (Zhang et al., 2018) studied the secondary metabolites of Salvia miltiorrhiza Bunge, growing in different environmental factors and topographical conditions,

55   found that the content of tanshinones was positively related to the content of aluminium (Al), magnesium(Mg) and barium(Ba) in the soil, negatively related to lead(Pb), and that total nitrogen and potassium(K) all contributed to the production of most phenolic acids.

   In some areas, plants produced have better nutritional value or higher active substance content, which is generally considered the "high-quality" production area of the crop, referred to in this paper as

60   the "Daodi-zone". As Guo et al. (Guo et al., 2013) analyzing the influence of environmental factors on jaundice secondary metabolites found that the zone from Chifeng, Inner Mongolia to Taibai, Shaanxi is suitable for the accumulation of secondary metabolites of Scutellaria baicalensis. However, what is the scope of the Daodi-zone? Is there a natural boundary? What environmental factors determine the boundaries of the Daodi-zone? These questions have not been interpreted. In the past, the definition of

65   Daodi-zone was judged based on human cultivation experience, and its scope was lacking in scientific experiments. Understanding the boundary of the Daodi-zone of plants is of great significance to the plant's rational introduction cultivation.

       Although climate factors dominate regional heterogeneity on large spatial scales, spatial distribution differences in soil elements may significantly impact crop quality differences at medium and

70   small spatial scales (Van der Putten et al., 2004;Santamaría et al., 2003). The geospatial variation of climate and soil factors is often inconsistent, resulting in different affect gradients (Macel et al., 2007). In the past, many studies have paid more attention on the variation of secondary metabolite content in large spatial scales or complex climate environments (Guo et al., 2013;Zhang et al., 2020), and there is an apparent lack of understanding of the heterogeneity of soil elements in the decision-setting of crop

75   yields at medium and small spatial scales in the same climate environment. Therefore, this study mainly discusses the spatial distribution of soil elements and their characteristic effect on the boundary of L.chuanxiong's Daodi-zone.

       Ligusticum chuanxiong Hort. is an important medicinal plant; its rhizomes have good efficacy in treating cardiovascular diseases, colds and arthritis (Chen et al., 2018). The Shiyang Town in Dujiangyan

80   City, Sichuan Province, has been recognized, at least from the Song Dynasty (960 A.D. to 1279 A.D.) in China, as producing the best medicinal value of L.chuanxiong, so is also known as L.chuanxiong's "Daodi-zone" (YIN et al., 2013), while L.chuanxiong outside Sichuan is produced as food. As a cultivated species, it is widely cultivated throughout China and cultivated in Japan and Korea. Hou (Hou, 2007) compared the quality of the same species L.chuanxiong in different cultivation region. He found

85   significant differences in the content of active ingredients, indicating that the influence of the cultivation environment on the quality of L.chuanxiong is apparent(Ma et al., 2009). It is evident that there is a typical "regional effect" in the quality of L.chuanxiong. Therefore, L.chuanxiong can be used as a representative plant to study the relationship between the accumulation of secondary metabolites and Geospatial variation in medicinal plants at the small and medium scale. We choose the Shiyang Town in

90   Dujiangyan City (the center-zone) as the starting point, along the same latitude to the east interval multi-point sampling obtain soil samples. By testing soil element composition and content to identify the heterogeneity of the soil elements and chemical properties in Daodi-zone; This study provides a reference for the scientific definition of other medicinal plants' Daodi-zone by revealing the natural geographical extent of L.chuanxiong's Daodi-zone, and this method is of great value to the plant's rational introduction

95   cultivation.

2 MATERIALS AND METHODS

2.1 Sample plots

The experiment was carried out in the fengdui village of Shiyang Town, Dujiangyan City (30˚50'10'N, 103˚40'03"E) and 280 kilometres eastwards at the same latitude (where the Tuojiang River separates E7

100   and E8, the Longquan Mountain chain separates E10 and E11; the Fujiang River separates E16 and E17; the Jialing River separates E23 and E24). Set Nine sample points in the center-zone and 28 sample points (one sample point per 10 km) in the eastern extension zone (Table 1, Figure 1). Since L.chuanxiong is cultivated with rice alternately,, the experiment was conducted from August to September 2017, i.e. before the cultivation of L.chuanxiong after the rice harvest. A total of 37 bulk soils (5-20cm depth) and

105   29 deep soils (150-180cm depth) were collected from rice paddies (100 m×100 m) of every sample points (3 biological replicates per sample). According to the HJ / T166-2004 guidelines (2004) to collect soil. To investigate the correlation between soil element changes and active ingredients. We planted L.chuanxiong in C0, E5 and E9 in May 2018, then collected samples of L.chuanxiong rhizomes, rhizosphere soil and bulk soil in mid-May 19.

110   Table 1 Information of the 37 sampling sites.

No.	Latitude	longitude	Altitude	No	Latitude	longitude	Altitude
C0	30˚50′10″	103˚40′0″	592	E11	30˚50′42″	104˚49′21″	471
CS1	30˚49′34″	103˚40′10″	599	E12	30˚50′05″	104˚54′49″	404
CS2	30˚47′36″	103˚40′08″	600	E13	30˚50′38″	105˚01′51″	387
CE1	30˚50′20″	103˚41′03″	595	E14	30˚50′41″	105˚08′21″	363
CE2	30˚50′10″	103˚43′12″	589	E15	30˚50′54″	105˚14′22″	357
CW1	30˚50′20″	103˚39′27″	574	E16	30˚50′34″	105˚20′43″	360

CW2	30°49′55″	103°36′50″	585	E17*	30°49′47″	105°26′40″	351
CN1	30°50′35″	103°40′04″	577	E18*	30°49′56″	105°33′42″	396
CN2	30°52′59″	103°40′15″	645	E19	30°49′30″	105°39′12″	577
E1	30°50′30″	103°46′02″	563	E20	30°50′26″	105°45′47″	535
E2	30°50′16″	103°52′51″	502	E21*	30°50′32″	105°52′06″	475
E3	30°50′16″	103°58′46″	482	E22	30°50′28″	105°58′32″	458
E4	30°50′22″	104°05′46″	465	E23	30°51′51″	106°04′32″	457
E5	30°50′52″	104°11′58″	440	E24	30°50′13″	106°10′55″	442
E6	30°49′20″	104°17′41″	435	E25*	30°49′22″	106°17′02″	422
E7	30°50′31″	104°23′54″	440	E26*	30°49′36″	106°23′22″	818
E8*	30°50′52″	104°30′33″	453	E27*	30°50′18″	106°29′54″	439
E9	30°50′37″	104°36′39″	424	E28*	30°50′22″	106°35′32″	450
E10	30°50′09″	104°12′52″	418		---		

The "*" marker is that deep soil sample has not been collected.

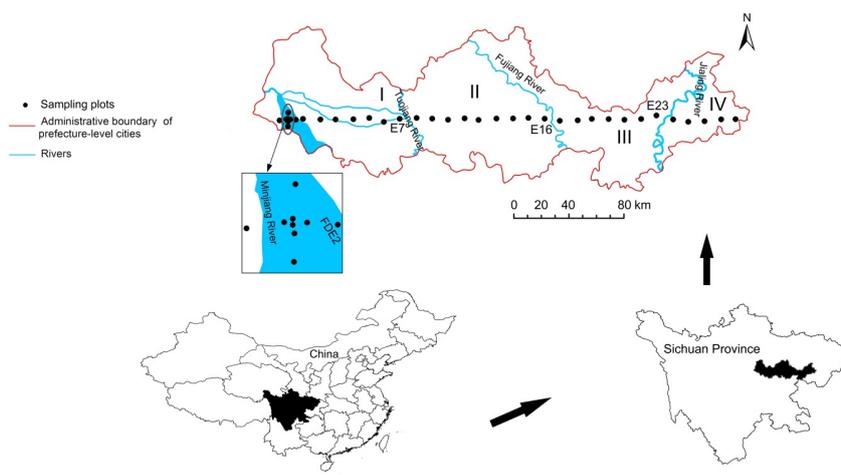



Figure 1 Sample plot. Locations of 37 sample points for this study. Maps generated by ArcMap.

2.2 Sample processing

115   Ground natural air-dried soil samples into three sizes (2mm, 0.15mm and 0.074mm) to determine soil

pH, organic matter and inorganic elements. Measuring pH in the ratio of 1:2.5 between soil and water

with the Spectrum 2105 SoilStik pH meter (Spectrum Technologies, Inc., Aurora, IL, USA). Organic

matter content determination uses $K_2Cr_2O_7$-H_2SO_4 to determine organic matter content (Bao, 2000). Then, soil powder was prepared as a stable disc pellet to detect the inorganic element concentrations with a

120 wavelength dispersive X-ray fluorescence (XRF) spectrometer S8 tiger (Fang et al., 2020).

For plant samples, rhizomes were washed, dried and grounded (0.25mm). Each powder was acidified by the addition of HNO3 and made up to 25mL with deionized water for the determination of element concentrations using a Nex10N 350x ICP-MS (Perkin Elmer, America), adding In115 as an internal element standard. Additionally, the contents analysis of senkyunolide A, senkyunolide I,

125 senkyunolide H, Ferulic Acid, ligustilide and coniferyl ferulate was achieved using analytical high-performance liquid chromatography (HPLC) with an Agilent 1200 series HPLC system. According to the fourth edition of the Chinese Pharmacopoeia, we detect the moisture content, total ash, insoluble acid ash, and leachate in plant samples (Wang, 2015).

2.3 Data analysis

130 All statistical analysis is performed in R 4.0.3(Team, 2013). Using the Shapiro-Wilk test to check the normal distribution of the data, using a one-way analysis of variance or Kruskal-Wallis test to assess the difference between the groups of samples, the Duncan test for two post-mortem tests, the p-value was corrected using Bonferroni. The multi-statistical analysis uses the Vegan 2.5-7 package (Oksanen et al., 2013). After converting inorganic element data into European distances, hierarchical clustering is

135 performed using unmeasured group arithmetic averages (UPGMA). PCA analysis and ANOSIM similarity testing based on clustering results. Using the simil function in the proxy package (Meyer and Buchta, 2020) to calculate the Eastern extension zone sample's similarity to elements in the center-zone, then linearly fit it with soil organic matter, pH, and altitude, and test its significance with a permutation test. Use the corrplot package to calculate the Pearson correlation coefficient of the active ingredients in

140 the roots of L.chuanxiong and the element content and plot the correlation(Wei et al., 2017). The formula for calculating the bioconcentration factors:

$$BCF = \frac{CRhizome}{CSoil} * 100\% \tag{1}$$

Where, in Eq. (1), BCF is an indicator of an element's tendency to accumulate in the organism. CRhizome is the Concentration of the element in the rhizome, CSoil is the Concentration of the element

145 in rhizosphere soil.

The formula for calculating the background change rate of soil chemical elements (Cheng et al., 2014) is

$$\Delta RCi = \frac{GBLi - GBGi}{GBGi} * 100\% \tag{2}$$

Where, in Eq. (2), ΔRCi is the rate of change of the natural background of element i, GBLi is the geochemical reference value of the i element, and GBGi is the geochemical background value of i

150   element. When $|\Delta RCi| \geq 100$, it indicates a significant increase or decrease in the i element, when $50 \leq |\Delta RCi| \leq 100$ indicates a significant increase or decrease in the i element, and when $0 \leq |\Delta RCi| \leq 50$ indicates an increase or decrease in the i element.

3 RESULTS

155   ### 3.1 Heterogeneity of Daodi and eastern extension zone

To compare soil heterogeneity in the center-zone and eastern extension zone, we sampled soil in the zone mentioned above at intervals of 10 km and analyzed soil elements' differences and physical-chemical characteristics in the study area.

3.1.1 Cluster analysis of soil element

160   To clarify the similarity of soil elements between the Daodi-zone and the eastern extension zone, we normalized the data of soil elements and clustered them using arithmetic average non-weight grouping, with all soil samples clustered into three groups, Cluster1, Cluster2, Cluster3 (Figure 2, Table 2). Among them, samples from all Daodi-zone are called center and are included in Cluster1. The E11-E14, E16-E19, which are geographically close to the center group, are clustered as Cluster 2; The E20, E21, E23

165   and E28 are far away from the center group as Cluster 3. The results show that the element similarity of most sample points is related to geographical location (Table 2). Still, a few sample points (E15, E22 and E24-E27) are far away from the center-zone but have similar element characteristics to the center group. Based on three clustered samples for PCA analysis, and ANOSIM similarity test results show significant differences between groups (Figure 3, P<0.05).

170   Among them, center-zone's pH is the lowest, 6.80. As the distance farther and farther from the center-zone, the pH value shows an upward trend. The center group also maintained the same organic matter content as Cluster 1 and Cluster2, but Cluster 3 had a higher OM of 38603.75 mg/Mg. In addition, the center group had only Cl, Br, La, and Th with lower average concentration levels than the other group samples. The center group is rich in P (1103.28 mg/Kg), K (23956.79 mg/Kg), Mg (12106.17 mg/Kg)

175   and Fe (38904.94 mg/Kg) compared to the elemental background values of Dujiangyan City (Tang et al., 2005). Mn (342.42 mg/Kg) and Ca (9623.46mg/Kg) are slightly missing from the required trace elements in the center group. Heavy metal elements Cu, Cr, Pb, Ni and Zn are all higher than the background value, 1.28 to 1.58 times the background value, and As is lower than the background value and 0.46 times the background value. These heavy metal elements meet the national standard requirements (GB15618-

180   2018): Cr≤300 mg.kg-1, As≤25 mg.kg-1, Ni≤1 00 mg.kg-1, Cu≤100 mg.kg-1, Zn≤250 mg.kg-1, Pb≤140 mg.kg-1. By comparing the characteristics of soil elements, we found that the center-zone is the characteristics of the alluvial soil (Huang and Jiang, 2010). Most of these element concentrations are higher than the background values of Chinese soil elements (layer A), mainly due to human intervention and the use of basic magma rock as their material source (Chen et al., 1991;He et al., 2006;Yang et al.,

185   2013). In addition, Ca in slightly acidic sandy soils can easily move downwards due to the humid and long-term flooded environment of the soil in the rice paddies. During the growth and development of rice, Mn (II.) and As (III.) (absorbed by iron oxide at the roots of rice) are absorbed (Chakravarty et al., 2002;Singh et al., 2011;Ma et al., 2018), resulting in concentrations of Ca, Mn and As being lower than the background values.

190   The concentration levels of a small number of elements in Cluster1 (excluding the center group sample) were higher than those in the center group, but the average increase was no more than 40.33%. In addition, the average concentration level of most elements in Cluster1 is higher than that of the Cluster 2 (up to 29.29%) and Cluster 3 (up to 43.40%). In the Cluster 2 and Cluster 3 groups, most elements were at similar concentration, but Ca, S and Cl in Cluster2 were higher, while Mg, Mn and As in Cluster3

195   were higher. It is worth noting that the concentration levels of Na in these two regions are about half the background value.

**Reordered dendrogram from
hclust(d = env.N, method = "average")**

36 sites
3 clusters

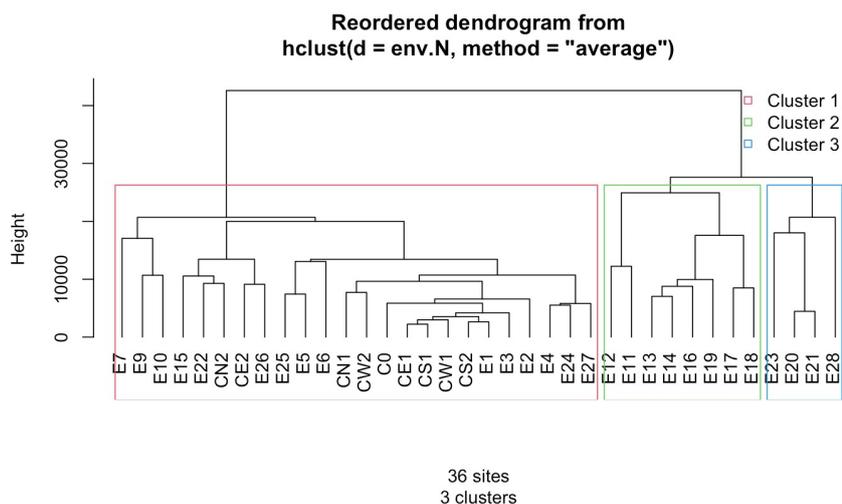



Figure 2 Clustering dendrogram. Based on the Euclidean distance metric, the inorganic elements of various points are hierarchically clustered using the unweighted pair group method with arithmetic mean method. Bulk soil samples include 9 (C0-CN2) in center-zone and 28 in Eastern Extension zone, for a total of 37 samples. cluster groups 3, Cluster1, Cluster2 and Cluster3, of which Cluster1 contains Center group samples (C0, CS1, CS2, CE1, CE2, CW1, CW2, CN1, CN2).

Table 2 The elemental content of the bulk soil

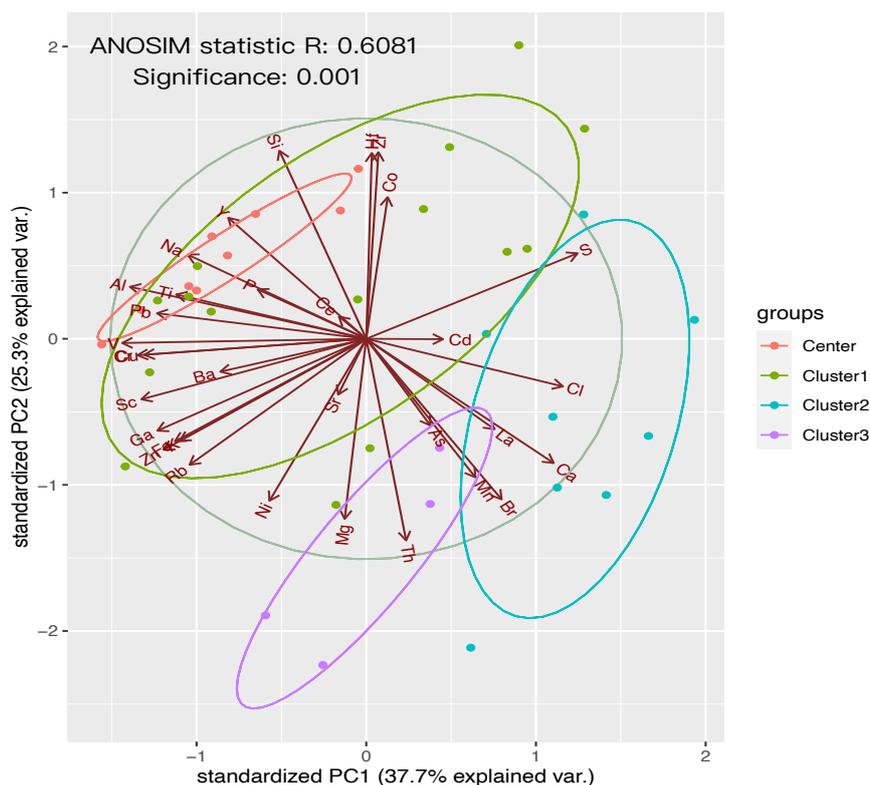
Element	Center	Cluster1	Cluster2	Cluster3	Bac.lev
Na	10459.26±1155.62	7652.59±1688.75	5411.11±541.86	4983.33±960.47	10200
Mg	12106.17±1057.35	11543.70±2376.04	12656.94±2000.55	15572.22±1811.32	7800
Al	86171.60±4520.84	81897.78±4712.05	72916.67±2196.53	78727.78±2470.44	66200
Si	282930.9±2778.61	284609.6±6488.69	266629.2±7231.95	265861.1±5281.43	--
P	1103.28±202.86	891.73±327.60	833.25±94.06	784.06±51.38	--
S	140.53±34.11	173.67±94.88	268.21±77.21	98.31±85.35	--
Cl	60.78±6.71	76.56±16.88	103.90±25.72	79.72±5.00	--
K	23956.79±1667.60	22264.44±3495.61	20802.78±1488.42	24316.67±1314.83	18600
Ca	9623.46±5400.28	11385.19±6357.36	50059.72±8362.27	33002.78±9059.39	15400
Fe	38904.94±1326.80	39697.04±4241.01	35011.11±2920.43	43041.67±3986.56	29400
Sc	14.60±0.56	13.74±1.56	12.68±0.99	14.05±1.36	--
Ti	4532.20±91.48	4523.73±238.73	4039.35±215.77	4474.05±64.90	3800
V	121.44±7.49	109.03±16.44	91.85±8.37	110.97±9.32	82.4

Cr	88.61± 5.69	84.18±10.57	73.06±2.21	85.68±9.12	61
Mn	342.42± 60.37	531.39±196.99	685.47±125.09	718.12±107.77	583
Co	29.26±5.51	25.62±7.63	23.63±5.58	20.23±1.52	12.7
Ni	38.38±1.43	38.16±4.23	38.27±1.90	46.49±7.06	26.9
Cu	35.78±3.38	32.43±6.91	25.30±2.38	32.27±5.35	22.6
Zn	94.93±16.34	90.72±25.90	75.31±16.40	103.03±10.74	74.2
Ga	20.36±1.44	19.68±1.76	18.12±0.95	20.56±0.91	17.5
As	5.13±2.37	8.59±3.14	8.64±2.79	12.56±2.46	11.2
Br	1.34±0.18	1.70±0.45	2.74±0.80	2.44±0.18	--
Rb	122.03±13.70	120.66±14.43	112.65±9.93	129.69±4.32	111
Sr	174.94±27.75	153.39±21.42	166.52±33.28	154.82±31.86	167
Y	29.64±0.38	29.11±1.74	26.89±1.42	27.53±0.63	22.9
Zr	232.37±11.43	235.65±23.10	212.59±23.85	197.91±16.77	256
Ba	615.20±43.29	569.89±63.79	580.19±52.89	528.50±43.12	469
La	35.95±2.61	39.63±4.23	41.44±2.01	44.77±2.23	--
Hf	6.60±0.32	6.58±0.46	6.10±0.50	5.79±0.45	--
Pb	34.53±5.01	30.90±4.25	25.71±1.68	29.37±1.63	26
Ce	73.50±3.09	74.88±4.52	71.10±2.78	73.31±2.26	68.4
Th	12.58±0.60	12.66±1.12	14.48±1.53	15.49±0.78	--
Cd	0.39±0.04	0.41±0.08	0.45±0.10	0.39±0.09	--
TN	1421.82±260.65	1385.24±213.84	1386.53±284.81	1862.40±256.81	--
Altitude	595.11±20.77	473.67±105.41	413.62±76.02	479.25±38.63	--
pH	6.80±0.76	7.38±0.71	8.10±0.13	7.91±0.08	--
OM	28535.00±3626.18	26656.11±5239.29	28036.25±7947.14	38603.75±5311.97	--

Samples of Center, Cluster1, Cluster2, and Cluster 3 are shown in figure 2. Inorganic element content is

205   in mg/kg. The value after "±" is the standard deviation, and all data is kept in two places. Bac.lev is the

background value of the reference chemical element in the Dujiangyan region. TN is total nitrogen and

OM is organic.

210   Figure 3 PCA plot. PCA analysis of inorganic element data in rhizome samples based on standardized
distance matrices. Samples of Center, Cluster1, Cluster2, and Cluster 3 are shown in Figure 2. The
ANOSIM similarity test showed significant differences among groups at 95% confidence levels (P<0.05).

3.1.2 The dissimilarity of nutritional content ratio in soil

215   We measured the ratios of Fe/Mn, Na/K, Zn/Cu, Ca/P, and K/Mg in the soil to determine soil nutrition's

heterogeneity in different regions (Fageria, 2001;Khan et al., 2009;Andrade et al., 2010;Senbayram et

al., 2016;Bornø et al., 2018). The Kruskal-Wallis test showed significant differences between groups

(Table 3), and the Duncan test showed no significant differences between the center-zone and the other

nutrient ratios of Cluster1, except for the significant differences of Ca/P ratios across all groups. There

220   was no significant difference in the Fe/Mn, Zn/Cu between Cluster1 and Cluster2, and no significant

difference in the Fe/Mn, Na/K, Zn/Cu, and K/Mg between Cluster2 and Cluster 3. Among them, the

Fe/Mn, Na/K and K/Mg of the Center group had the highest ratio compared to the other three expansion

zone samples, increasing by an average of 44.1%, 38.6% and 13.3%, respectively, while the Ca/P and Zn/Cu ratios were the lowest, with an average decrease of 369% and 13.3%, respectively.

225

Table 3 The ratio of nutritional element

	CENTER	CLUSTER1	CLUSTER2	CLUSTER3
FE/MN	116.82±20.88 a	82.52±26.92	52.40±9.13 a	61.10±11.87 a
NA/K	0.44±0.07 a	0.34±0.05 b	0.26±0.03 ab	0.21±0.05 ab
ZN/CU	2.64±0.32 a	2.80±0.52	2.95±0.44	3.22±0.21 a
CA/P*	8.38±3.10	14.52±9.15	61.24±0.44	42.14±10.92
K/MG	2.00±0.24 a	1.96±0.24 b	1.67±0.18 ab	1.57±0.11 ab

The table shows the ratio of the nutritional element, and the sample composition of Center, Cluster1, 2, and 3 are shown in figure 2. Using the Kruskal-Wallis test and the Duncan test, detect the difference between groups; the confidence level was 95%. The lowercase letter a is a significant difference between the Center group sample and the rest of the sample, the lowercase letter b is a significant difference between Cluster 1 and Cluster 2 or Cluster 3, and "*" means there is a significant difference among all groups in Ca/P ($P<0.05$).

3.1.3 The similarity analysis of soil elements and linear regression analysis

235 Calculate the element similarity between the Daodi-zone and the samples in the eastern extension zone. We found that the elemental similarity coefficient of the sample decreased at the beginning the further away from the Daodi-zone, reaching the lowest point at E13 (0.53), and then gradually recovered, reaching the highest value at E27 (0.78) (Table 4). The content of altitude, pH and soil organic matter was linearly fitted with similar coefficients, and the permutation test showed that the similar coefficients

240 of soil element spectrum were positive correlation with altitude and negatively correlated with pH and OM (Figure 4, $P<0.05$).

Table 4 The similarity coefficient of the element

sample	S.I	sample	S.I	sample	S.I
E1	0.92	E11	0.72	E20	0.72
E2	0.91	E12	0.68	E21	0.75
E3	0.89	E13	0.56	E22	0.74
E4	0.87	E14	0.67	E23	0.64
E5	0.83	E15	0.73	E24	0.73
E6	0.77	E16	0.61	E25	0.75
E7	0.67	E17	0.63	E26	0.75
E9	0.68	E18	0.65	E27	0.80
E10	0.63	E19	0.66	E28	0.66

Based on the "simil" function in the proxy package, we calculate the similarity coefficient between the

245   samples in the eastern extension zone and the soil elements in the center-zone.

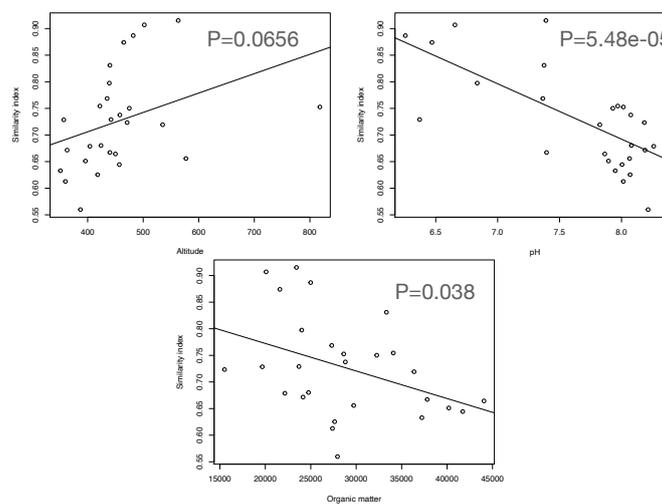



Figure 4 Linear regression. Analyzing the linear regression between elemental similar coefficients and altitude, pH, and organic matter at a confidence level of 95% (P<0.05).

3.2 Soil elements shape the characteristics of the absorption of elements of L.chuanxiong and the accumulation of active ingredients

3.2.1 Analysis of the active ingredients and elements

To verify the effect of soil element changes on active ingredients, we chose to conduct field cultivation experiments at E5 and E9 points, which belong to different cluster trees in Cluster1, using C0 sample points as a control group. Then sample rhizome, rhizosphere soil and bulk soil in mid-May 19. We measured the content of ingredients and elements in the rhizome (Table 5). The one-way analysis of variance between groups showed significant differences in the content of ligustilide and moisture. Then Duncan's test shows significant differences in the ligustilide and moisture between the C0 and E9 sample points ($P<0.05$), with no significant difference with E5. Besides, correlation analysis of the active ingredients and elements in the rhizome showed a significant negative correlation between Mn and Coniferyl Ferulate and Ligustilide, a significant positive correlation with Ferulic Acid, and a significant positive correlation between Se and Senkyunolide I, Senkyunolide H and Ligustilide, and a significant positive correlation between Sr and Ligustilide (Figure 5, $P<0.05$).

Table 5 The content of ingredients and elements in the rhizome

	C0	E5	E9
Content			
Senkyunolide A	0.93±0.20	0.91±0.28	0.81±0.16
Senkyunolide I	0.013±0.004	0.016±0.006	0.011±0.003
Senkyunolide H	0.004±0.001	0.004±0.002	0.003±0.001
Ferulic Acid	0.018±0.003	0.019±0.005	0.10±0.14
Coniferyl ferulate	0.45±0.088	0.48±0.14	0.24±0.20
Ligustilide	2.26±0.20 a	2.19±0.41	1.75±0.03 a
Alcohol soluble extract	29.77±2.14	30.60±2.56	29.10±0.90
Total ash	4.16±0.40	3.67±0.27	4.28±0.42
Acid insoluble ash	0.55±0.15	0.37±0.15	0.35±0.064
moisture	9.53±0.88 a	8.32±0.38	8.03±0.96 a
Element			

Zn	28.24±7.44	29.75±5.90	28.93±2.19
B	18.008±1.87	15.81±1.26	14.03±0.59
Ca	3009.31±597.93	1920.21±203.68	2038.10±253.43
K	7703.98±885.49	6665.97±456.83	9302.22±1572.60
Na	1425.50±370.70	1470.54±198.23	559.26±348.79
Mg	2266.99±287.51	2096.22±184.49	1944.53±138.37
P	4267.74±825.86	4554.83±400.53	4793.27±183.55
AL	135.35±86.36	26.00±8.40	25.21±0.53
Ti	3.95±1.74	1.64±0.36	1.65±0.17
V	0.31±0.20	0.062±0.016	0.062±0.012
Cr	0.31±0.16	0.14±0.086	0.083±0.011
Mn	25.67±5.06	25.64±5.16	37.75±9.17
Fe	111.00±48.96	49.98±7.74	39.76±1.94
Co	2.34±1.20	4.05±5.06	2.64±1.08
Ni	2.05±0.74	1.71±0.46	1.74±0.50
Cu	8.84±1.44	9.76±3.02	12.10±0.88
Ga	1.11±0.44	0.75±0.13	0.64±0.27
As	0.15±0.043	0.064±0.017	0.051±0.012
Se	0.044±0.018	0.055±0.032	0.012±0.011
Rb	7.48±3.00	9.73±1.91	3.62±1.72
Sr	21.19±2.97	14.10±1.71	9.26±0.24
Y	0.072±0.036	0.014±0.003	0.026±0.012
Zr	0.084±0.03	0.024±0.004	0.026±0.006
Mo	0.15±0.08	0.12±0.052	0.076±0.021
Cd	0.32±0.21	0.18±0.10	0.11±0.025
Ba	12.63±5.09	7.86±1.36	7.38±3.21
La	0.11±0.055	0.024±0.006	0.033±0.019
Ce	0.15±0.089	0.035±0.01	0.049±0.024
Nd	0.068±0.041	0.015±0.004	0.026±0.014

Pb	0.51±0.21	0.28±0.13	0.27±0.13

The active ingredients and regular component units are %, and the inorganic element content units are mg/kg. The value after "±" is the standard deviation and keep all data in two places. The lowercase letter "a" indicates a significant difference in the belief level of an ingredient between groups at 95%. The P-value is corrected using Bonferroni (P<0.05).

270

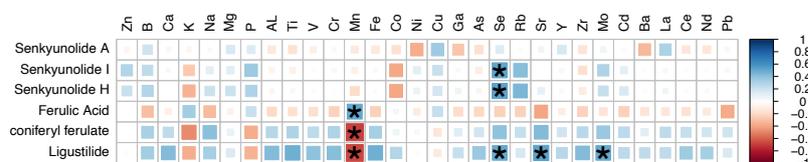



Figure 5 Correlation analysis. Pearson correlation analysis of active ingredients and elements in the rhizome, "*" indicates a significant difference at a 95% confidence level (P<0.05).

3.2.2 Analysis of the characteristics of soil and rhizome absorption element

275   PCA analysis of the composition and content data (Table 6) of bulk soil, rhizosphere soil and rhizome at each sample points, and the ANOSIM test shows significant differences among groups (Figure 6, P<0.05). Moreover, as the distance farther and farther from C0, pH in the rhizosphere and bulk soil samples shows an upward trend, while OM shows a downward trend.

To understand the characteristics of the absorption of different elements by the L.chuanxiong's

280   rhizome at three sample points, we calculated the bioconcentration factors(BCF) of elements in the rhizome (Table 6). The highest BCF (2.24-7.4) of P in the rhizome was found in all three sets of samples, with a BCF average of 0.08-0.099 for other elements. It is worth noting that there is no obvious correlation between the content of Zn in the rhizome and the content in the rhizosphere soil, even though the content of Zn (57.8 mg/Kg) in the rhizosphere soil at E9 points is significantly reduced compared to

285   the other two points, but at this time the content of Zn (28.93 mg/Kg) in the rhizome is still close to the

concentration of the other two points (BCF is the highest, 0.5). Besides, we observed a negative

correlation between Ca and Cu content in the rhizome and content in the rhizosphere soil. The Ca content

of the rhizosphere soil in C0 points was the lowest compared to E5 and E9 (7737.04 mg/Kg), while the

average content of Ca was the highest in the rhizome at C0 points (3009.31 mg/Kg, BCF is also the

290   highest, 0.39). Cu content in E9-point rhizosphere soil was the lowest compared to C0 and E5 (30.73

mg/Kg), while the average content of Cu in E9 rhizome was highest (12.10 mg/Kg, BCF is also the

highest, 0.39).

Table 6 The elemental content of rhizosphere and bulk soil

	C0N	C0R	E5N	E5R	E9N	E9R
Na	9761.90±379.29	10062.96±582.96	9402.22±442.66	9584.44±387.89	4566.67±732.07	4616.67±1139.06
Mg	9988.10±550.48	10125.93±434.54	11575.56±939.56	11331.11±745.30	8254.76±1355.81	8666.67±1991.41
Al	82252.38±694.12	80866.67±396.51	81560.00±4819.69	82288.89±3469.89	78290.48±5217.91	79308.33±7932.01
Si	292583.3±2900.21	291281.5±1997.71	289817.8±6451.98	288882.2±4545.45	293026.2±10751.79	293091.7±16322.97
P	1552.33±71.09	1905.22±254.27	945.76±483.53	1243.22±396.46	628.29±61.59	647.83±122.56
S	171.86±24.20	186.89±22.51	139.22±29.50	152.07±36.25	214.52±47.86	234.58±102.74
Cl	63.00±3.76	82.37±9.14	76.49±19.58	115.56±35.97	101.45±24.96	153.75±39.69
K	22378.57±1030.94	22688.89±932.74	22300.00±1279.94	22800.00±925.65	15742.86±2000.62	17104.17±2810.44
Ca	6547.62±637.95	7737.04±1234.28	8666.67±584.59	8144.44±657.88	11602.38±5093.32	13133.33±6857.97
Fe	37178.57±1351.84	36374.07±805.67	43906.67±4110.46	41424.44±3526.42	41526.19±5023.22	43266.67±6758.65
Sc	13.64±1.07	13.01±0.98	15.39±1.39	14.66±1.31	13.47±1.75	14.54±2.36
Ti	4648.05±72.97	4545.11±72.93	4815.68±188.42	4680.60±152.70	4609.77±196.05	4674.25±253.11
V	116.20±3.68	113.36±1.85	130.65±9.56	125.08±7.90	108.70±11.82	112.56±16.09
Cr	85.82±2.42	83.25±1.65	109.49±6.40	101.33±8.48	82.78±7.37	86.01±9.78
Mn	339.98±27.44	345.78±24.88	668.54±236.41	566.80±222.76	701.79±85.58	645.35±105.16
Co	31.53±3.58	34.40±9.59	27.32±2.21	29.53±5.13	32.22±7.33	40.01±22.72
Ni	35.69±2.09	35.71±1.64	47.35±6.22	42.94±5.46	41.77±4.07	44.27±5.10
Cu	36.98±1.35	37.08±1.49	40.32±1.75	40.26±1.71	30.78±3.09	30.73±3.74
Zn	95.02±5.00	98.84±9.49	109.78±11.18	107.31±14.86	66.02±16.41	57.80±41.89

Ga	19.18±0.63	19.29±0.60	20.11±1.58	20.20±0.94	18.28±2.34	19.94±3.58
As	5.64±0.67	4.64±1.77	7.42±0.40	5.98±1.53	15.28±2.30	13.78±5.38
Br	1.26±0.15	1.41±0.34	1.77±0.73	1.66±0.72	1.36±0.29	1.26±0.62
Rb	121.32±5.31	121.44±5.82	124.14±9.11	123.73±5.79	103.83±14.45	110.15±19.05
Sr	170.39±15.85	177.76±19.42	164.80±5.63	166.00±5.95	96.65±7.97	99.66±11.17
Y	28.74±0.57	28.53±0.49	28.54±1.02	28.78±0.69	35.36±1.41	34.24±1.25
Zr	247.83±12.67	242.94±8.95	205.87±7.43	217.86±13.28	277.37±38.78	257.08±47.52
Ba	600.63±26.14	593.47±22.20	620.35±27.90	616.08±22.53	496.58±56.47	511.20±74.30
La	32.69±3.21	35.80±2.27	35.62±3.65	35.80±4.11	44.51±2.50	45.45±3.40
Hf	7.05±0.38	6.90±0.28	6.03±0.25	6.30±0.45	7.55±0.96	6.98±1.24
Pb	36.89±1.87	35.56±2.44	34.75±3.99	34.93±4.37	29.79±2.12	28.55±2.18
Ce	71.95±3.52	74.12±5.60	72.50±5.65	72.68±6.17	84.36±3.53	86.17±4.10
Th	11.85±0.77	12.50±0.70	11.90±1.65	12.22±0.85	13.33±1.51	13.84±3.15
Cd	0.35±0.14	0.38±0.10	0.39±0.15	0.44±0.042	0.31±0.15	0.39±0.049
TN	1627.78±0.0046	2148.23±0.016	1365.80±0.14	1673.47±0.035	1318.25±0.02	1502.62±0.15
OM	2.78±0.06	3.66±0.24	2.35±0.67	2.78±0.62	1.82±0.52	2.02±0.33
pH	5.97±0.14	6.46±0.61	6.51±0.50	6.02±0.43	7.55±0.50	7.21±0.66

295   The inorganic element content units are mg/kg. The value after "±" is the standard deviation, keep all data in two places. C0N is the bulk soil of the C0 sample, C0R is the rhizosphere soil of the C0 sample, E5N is the bulk soil of the E5 sample, E5R is the rhizosphere soil of the E5 sample, E9N is the bulk soil of the E9 sample, and E9R is the rhizosphere soil of the E9 sample.

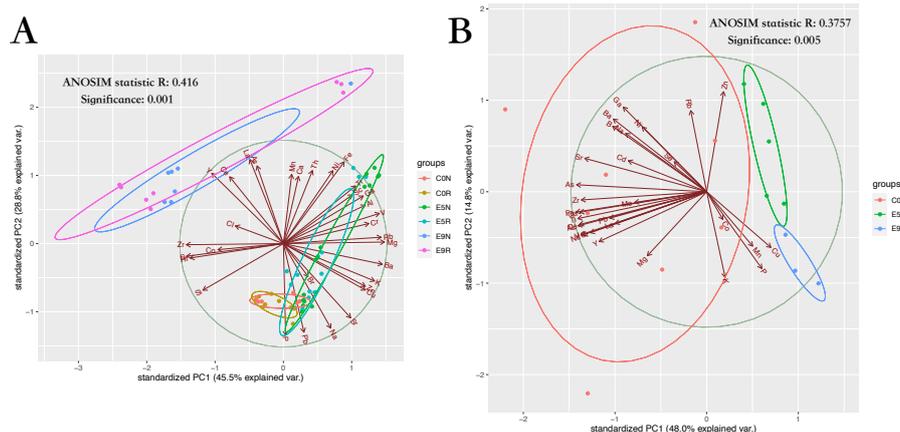



Figure 6 PCA. PCA based on a standardized distance matrix of elemental data. A is the soil sample, C0N is the bulk soil of the C0 sample, C0R is the rhizosphere soil of the C0 sample, E5N is the bulk soil of the E5 sample, E5R is the rhizosphere soil of the E5 sample, E9N is the bulk soil of the E9 sample, and E9R is the rhizosphere soil of the E9 sample. B is a sample of the rhizomes of Ligusticum chuanxiong Hort. The ANOSIM similarity test showed significant differences between groups at a 95% confidence level ($P < 0.05$).

Table 7 bioconcentration factors

	C0	E5	E9
Na	0.14	0.15	0.12
Mg	0.22	0.18	0.22
P	2.24	3.66	7.4
K	0.34	0.29	0.54
Ca	0.39	0.24	0.16
Fe	0	0	0
Ti	0	0	0
V	0	0	0
Cr	0	0	0
Mn	0.07	0.05	0.06
Co	0.07	0.14	0.07
Ni	0.06	0.04	0.04

Cu	0.24	0.24	0.39
Zn	0.29	0.28	0.5
Ga	0.06	0.04	0.03
As	0.03	0.01	0
Rb	0.06	0.08	0.03
Sr	0.12	0.08	0.09
Y	0	0	0
Zr	0	0	0
Ba	0.02	0.01	0.01
La	0	0	0
Pb	0.01	0.01	0.01
Ce	0	0	0

3.3 The rate of change in the background of chemical elements

The background value of the chemical elements of the soil is controlled by the soil parent material, which
reflects the natural geological background. We calculated the background change rate of chemical
elements in center-zone and the eastern extension zone (Figure 7). Results showed significant increases
in Al, Fe, Mg, Si, Ca, K, and Na in the soil. Besides, the background change rate of other chemical
elements in the soil in the center zone remained relatively small variation, and only content of Mn
significantly changed, decreasing by 55.46%. P and Cu in the eastern extension zone had a significant
increase by 117.1% and 67.67%, respectively, and the Ni and Mo fluctuated mainly with the geographical
coordinates, indicating a specific geographical heterogeneity. Throughout the study area, the overall
content of other potentially harmful and rare earth elements increased in the soil, excluding Ba, Co and
La. Besides, abnormal increases were found in Zn at E15, As at E19 and Th at E10. It is explained that
there are changes in soil element composition characteristics in the cultivation of L.chuanxiong, which
is mainly due to plant cultivation and its artificial intervention, such as fertilization, irrigation.

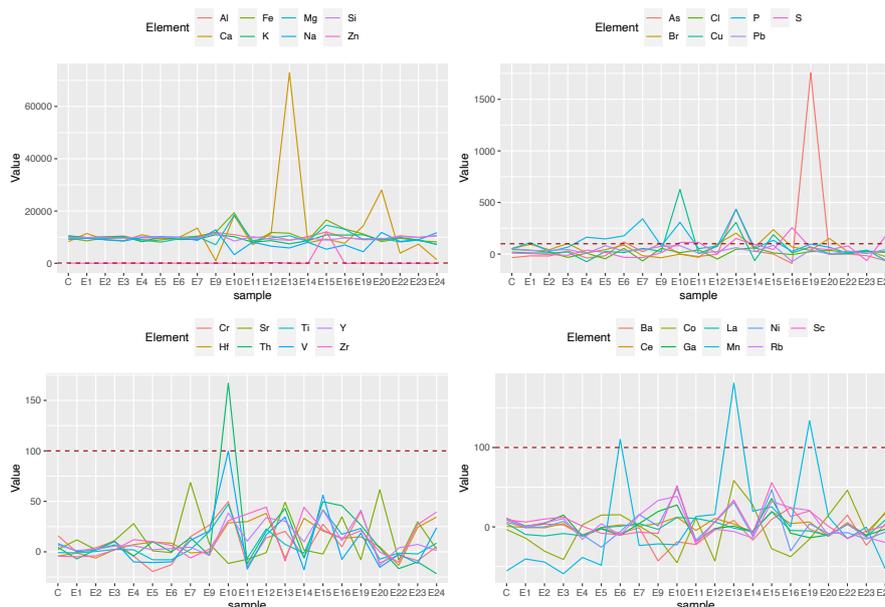



Figure 7 The change rate of background chemical elements. C is the average value of the center-zone. The red dotted line is a change rate of 100, and exceeding the red dotted line indicates a significant increase in the element's content.

4 DISSCUSION

4.1 The geographical heterogeneity of soil elements in center and eastern extension zone

Since L.chuanxiong is a medicinal plant with typical regional effects, we investigated the variation of elements in the bulk soil in the traditional central zone of L.chuanxiong (Shiyan Town, Dujiangyan City) and the eastern extension zone. The clustering analysis of soil elements shows that there is significant regional heterogeneity in the study zone and can be divided into at least three cluster groups (Cluster1 includes all central zone samples and E1 to E10, E15, E22, E24 to E27; Cluster2 includes E11 to E14, E16 to E19; Cluster3 includes E20, E21, E23, E28). Moreover, some samples of the eastern extension zone are very close to the central zone's soil element features, indicating that the soil characteristics of the Daodi-zone are not limited to the existing region.

The Eastern Extension zone covers the plain areas of western Sichuan and the hilly and low mountain areas of central Sichuan (Figure 1, E1 to E28). Rock bases in this region are diverse due to the deformation and fracture of geological structure caused by topographic uplift and deposition (Liu et al., 2012). Most of the elemental concentrations in Cluster1 are higher than Cluster2 and Cluster 3. On the

one hand, some samples in Cluster1 are close to the center-zone (including E1 to E10), and their soil

340   sources are consistent with the central region, characterized by the high maturity of paddy soil and the

magmatic rock as a raw material (Tang et al., 2005). On the other hand, cluster1 has samples such as E24

to E27 that are geographically far from the center-zone, which may be due to the short history of

cultivating L.chuanxiong in the local region(PENG et al., 2007). Local farmers influence the proportion

structure of the original nutrients in the soil by using the soil management methods of the L.chuanxiong,

345   which causes the local soil to be similar to the elements of the central region to some extent.

Besides, E10 sample points and subsequent sample points interval Longquan Mountain chain, due

to the laboratory early investigation (Fang et al., 2020) of soil elements with the central zone as the

starting point along the longitude south of the change characteristics, found that S1 to S6 sample points

were similar with the central region. The S6 and subsequent sample points are also separate in the

350   Longquan Mountains, so the results of the two studies suggest that the composition of soil elements will

change significantly due to the elevated terrain, indicating that the range of L.chuanxiong' Daodi-zone is

likely to be related to the distribution height of the Longquan Mountain chain.

The soil parent material of sample points in Cluster2, which extends mainly from the Tuojiang

River to the Fujiang River, is mainly composed of calcareous purple sandstone and is rich in calcium

355   (Zheng-An et al., 2010), which interprets that the average Ca content in Cluster 2 is the highest in all

groups. Cluster3, similar to Cluster2, is also mainly affected by calcareous purple sandstone, so Ca

concentration levels are also high. The linear regression analysis shows that the center-zone's elemental

similarity coefficient is mainly positively related to altitude and negatively related to pH and OM.

4.2 The regional heterogeneity of soil elements affects the accumulation of active ingredients

360   The central zone where C0 located is a recognized Daodi-zone of L.chuanxiong. The samples' soil

elements in Cluster 2 and Cluster 3 are significantly different from the central region, but samples in the

eastern extension zone in Cluster 1 are more similar to the central zone. Therefore, to screen the

characteristic soil elements that will significantly affect the accumulation of metabolites in the rhizomes

of L.chuanxiong, we selected E5 and E9 in Cluster1, which belong to two differnet cluster trees, and C0

365   in the central zone to conduct field cultivation experiments on L.chuanxiong (in cluster trees, E5 is closer

to the sample in the central-zone, and E9 is farther away). We found the ligustilide was significantly

higher than E9 in the rhizome sample at the C0, and there was no significant difference with E5, so was

the moisture content in the rhizome. Since the report shows that Zn is not conducive to the synthetic accumulation of Ligustilide and Senkyunolide A, we observed that Zn's concentration in rhizomes of the

370   three sites were close to each other, but Zn's BCF was the highest in the E9 sample points. We speculated that during the process of L.chuanxiong strengthen Zn's absorption, resulting in the uneven distribution in the rhizome at E9, thus affecting the synthesis ability of secondary metabolites in the rhizome(Chen et al., 2020). Therefore, high Zn's BCF of L.chuanxiong at E9 may be one reason why secondary metabolites' content is different from C0 and E5.

375   In addition, the study showed that the synthetic accumulation of secondary metabolites in L.chuanxiong's rhizome was positively correlated with the soil organic matter(Fan et al., 2013). In this experiment, the soil organic matter content in C0 and E5 was close, and the E9 was lowest, whereas the content of most active ingredients in C0 was similar to E5 but higher than E9. It is indicated that the more similar the characteristics of the soil elements, the more similar the characteristics of the content of

380   secondary metabolites composition obtained by cultivation.

Since inorganic elements also play an essential role in plant metabolism(Zhang et al., 2015;Wang et al., 2020;Abou-Arab and Abou Donia, 2000;Wen-Lan et al., 2015), we observed a significant positive correlation between Se and Senkyunolide I, Senkyunolide H and Ligustilide in the rhizomes of L.chuanxiong, possibly because Se improves the overall physical and chemical properties of plants,

385   improves secondary metabolites, and helps plants overcome the stress of Cr in the soil(Handa et al., 2019). Besides, Mn in the rhizome was significantly negatively associated with Conferyl ferulate and Ligustilide, positively related to Ferulic Acid, and Sr and Ligustilide in the rhizome were positively related significantly. This may be since heavy metals play the role of non-biological stimulates in biosynthesis, affecting plants' secondary metabolic processes (Lajayer et al., 2017a). Moreover, reports

390   indicate that medicinal plant with invigorating blood circulation, such as L.chuanxiong, is usually rich in K, Na and Mg, but deficient in Mn(Wu, 2017). Among them, Na and K are essential for acid-base balance. K is responsible for dilating blood vessels, while Mn at high concentrations can cause blood vessels to constrict (He and MacGregor, 2008;Aschner and Aschner, 2005). Mg is closely related to the activation of enzymes and calms the nervous system (Jahnen-Dechent and Ketteler, 2012). K and Mg in medicinal

395   plants also play a detox and live blood role. In this study, the average concentration of soil nutrients such as Na, Mg and K in center zone were significantly higher than the background values, and Na, Mg and

K were significantly higher than E9 samples in the rhizosphere and bulk soils of E5 samples, so this may be a condition for the production of high-quality L.chuanxiong.

4.3 Increased risk of heavy metal pollution in production zone

400 During the long-term cultivation process, the content and composition of elements in the soil usually change. Besides, climate change and industrial activities can also impact the soil elements. By analyzing the spatial heterogeneity of deep soil elements in the study zone, we evaluated soil elements' changing characteristics in the central and eastern extension zone. In the rice paddies, soil samples in the center-zone, Cu, Cr, Pb and Zn, were all higher than the local soil background values, although the soil's heavy

405 metal elements did not exceed the standard. The background change rate of chemical elements also shows that the content of most potentially harmful elements and rare earth elements has increased, indicating that the soil environmental safety in the study zone is gradually decreasing (Ren et al., 2005;Yang et al., 2018b). It is suggested that we need to strengthen the environmental protection work in the L.chuanxiong planting area to avoid the decline of safety and the reduction of the available area in the production zone.

410 Based on the above results, we believe that there is a geo-boundary of the suitable elemental zone of L.chuanxiong, which can indicate the natural boundary of the high-quality L.chuanxiong's Daodi-zone. In pre-laboratory studies, Fang et al. found that the soil elements extending 60 km southward from the center-zone (Dujiangyan Shiyang Town) to the territory of L.chuanxiong were the Daodi-zone of L.chuanxiong, and found that K, Mg, Mn and Rb could be used as soil characteristic factors (Fang et al.)

415 to evaluate the production zone of L.chuanxiong. In this experiment, we believe that the area extending 55 kilometres eastward along the latitude from the center-zone (Dujiangyan Shiyang Town) is the L.chuanxiong's Daodi-zone. Soils in this area are characterized by weak acidity, high Na, Al, P, Cu, Zn, Fe/Mn (116.82±20.88), Na/K (0.44±0.07) and K / Mg (2.00±0.24) values, while Zn/Cu (2.64±0.32) and Ca/P have lower values (8.38±3.6). Comparing the soil characteristics of the Daodi-zone in the two

420 studies, they are rich in Na, Mg, K and low Mn. Combined with the area's topography, the natural boundary of L.chuanxiong's Daodi-zone is located on the west and north sides of the Longquan Mountains (southwest to northeast) in both directions. The two studies did not cover the west and northerly aspects of the L.chuanxiong Daodi-zone because the west and north of Dujiangyan are right in the intersection of the Sichuan Basin and the surrounding mountains. Therefore, we think that

425 L.chuanxiong's Daodi-zone is likely to be plain, including Dujiangyan as its starting point, extending

eastwards and southwards to about 60 km in the Longquan Mountains, respectively. Although all the study areas span the Tuojiang, Fujiang and Jialing rivers, they have not had a significant impact on the boundaries of the L.chuanxiong's Daodi-zone.

430   This study successfully verified that the Daodi-zone has natural boundaries, and it is feasible to identify the suitable growth area of plants based on the characteristic soil elements. We also suggest that the high-quality production zone for certain economic plants is limited to a particular region and that the dominant factor in this phenomenon is likely to be a specific combination of soil elements. This study also provides a favourable environmental zoning basis for other economic plants with smaller areas of origin.

435

Data availability

All data are included in the paper.

Author contributions

ZY, DH and QF designed the research. QF, HL, LK, QS performed the research. HL analysed the data,

440   and HL, HW, DH, XZ and GH wrote the paper.

Competing interests

The authors declare that they have no conflict of interest.

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