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Correlations between Light Rare Earth Elements in Soil and Navel Orange Tree System in Gannan Area

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Abstract The total content of light rare earth elements (LREEs) in the soil of navel orange orchards of Gannan area is greater than that of heavy rare earth elements (HREEs). Appropriate content of LREE can not only promote the growth of navel oranges, and it is also conducive to human health. On the basis of exploring the correlations between the content of LREE in the soil of navel orange orchards of Gannan area and the contents of LREE in navel orange leaves and fruit, the influence mechanism of LREEs on the quality of navel oranges was revealed. In this study, with two Newhall navel orange orchards with different soil LREEs background levels in Xinfeng County as the research object, the changes in the content of LREE (lanthanum, La; cerium, Ce; praseodymium, Pr; neodymium, Nd) in leaves and fruit of navel orange at different growth stages were analyzed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), and the correlations between the content of LREE in the soil, navel orange leaves and navel orange fruit were studied. The results showed that the contents of the four kinds of LREEs in the soil ranked as Ce > La > Nd > Pr, and there were significant differences among them ($P<0.01$). Navel orange leaves and fruit have selective and heterogenic absorption for LREEs. At different growth stages, La showed the highest accumulation amount in the leaves and fruit of navel orange; and the content of LREE in the leaves of navel orange increased first and then decreased, while that in the fruit of navel orange showed continuous decrease. During the migration of LREEs from soil to leaves to fruit, the content of LREE decreased rapidly as the migration distance increased. The accumulation amount of LREE in navel orange was positively correlated with the content of LREE in the soil. The correlation between the content of LREE in the leaves and fruit of navel orange was greatest. Among the four kinds of LREEs, the correlation of La was greatest, followed by Ce, indicating that the accumulation amount of LREE in the navel orange body was affected by the element types.

Key words Light rare earth element, Navel orange, Soil, Correlation

1 Introduction

China is the most abundant country in the world for rare earths. The application of rare earths in agriculture has received national attention and support and has attracted world's attention[1]. Citrus fruits production ranks second in the world and also in China in the past years. Among them, navel orange plays an important role in citrus fruits in China. The major cultivar Newhall navel orange (*Citrus sinensis* (L.) Osbeck 'Newhall') accounts for more than 85%. Navel orange and rare earth are two major economic pillar industries in Gannan area of Jiangxi Province, enjoying the reputation of "the hometown of navel oranges in the world" and "the kingdom of rare earths". In the mining area of rare earths in Gannan, the soil rare earths content is 396.00 – 2 314.00 mg/kg, and the minimum content is more than two times the average content of soil rare earths in China[2]. It is generally considered that the soil with high-content rare earths is beneficial to the growth of fruit trees and can improve fruit quality and yield[3-4]. The effect of light rare earth elements (LREEs) is greater than that of heavy rare earth elements (HREEs)[5-6]. In the soil of navel orange orchards in Gannan area, the total content of LREEs is much higher than that of HREEs[7-8]. The contents of various organic nutrients in navel orange planted in the area with high rare earth background level, especially in the rare earth mining area, were slightly higher than those planted in area with low rare earth background level, with excellent taste quality[9]. However, Jin Shulan *et al.*[10] found that the rare earth contents in 10 kinds of crops in Gannan area were 1.04 – 78.57 mg/kg, exceeding the upper limit of rare earth elements (0.70 mg/kg) for the Chinese vegetable hygiene standards. The average daily intake of rare earth elements in the mining area is 295.33 μg/kg, much higher than the critical value of the subclinical damage dose of rare earth elements to human body. From the perspective of safety and long-term interests, navel orange growing in the soil with high rare earth content has the possibility of harming human health through the food chain for its high rare earth content.

Currently, a large number of studies have been conducted on the safety assessment of crops growing in high rare earth soils in Gannan[11]. There are many reports on the determination method of rare earth content in navel orange orchard soil and navel orange plants[12-14]. The inductively coupled plasma-atomic emission spectrometry (ICP-AES) established by Liu Xia *et al*[15], is suitable for determination of soil rare earth elements for wide linear range, low detection limit, high sensitivity and high precision (1.34% – 4.07%; *RSD*, $n=5$). Many studies have been conducted on the distribution characteristics of soil rare earths from the aspects of geochemistry[16-17], environmental chemical fea-

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tures[18], effective distribution[19], occurrence pattern[20] and migration characteristics[7, 21]. Although the correlations between the contents of organic nutrients in the fruit of Gannan navel orange and the content of rare earths in the soil have been studied preliminarily[9, 22], the distribution of rare earth elements in the soil and navel orange plants of Gannan area, the contribution rate of soil rare earth elements to the quality of navel orange, or the influencing mechanism of rare earth elements on the quality of navel orange is not yet clear. To this end, two navel orange orchards were selected from Xinfeng County in Gannan area with high soil rare earth background level, and the correlations between soil rare earth content and navel orange leaf and fruit rare earth contents were discussed based on analyzing the changes in soil rare earth content and the distribution characteristics of rare earth elements in navel orange leaves and fruit at different growth stages, so as to understand the correlation between soil rare earth content and navel orange plant rare earth content in the area with high soil rare earth background level, provide a theoretical basis for the in-depth study of the mechanism of the influence of rare earths on the quality of navel orange, and provide a scientific basis for the cultivation of high-quality navel orange in Gannan area.

2 Materials and methods

2.1 Materials

2.1.1 Selection of navel orange trees. Two Newhall navel orange orchards, in an area of 10 ha, were selected from Longshe Village (115°0′2 747″ E, 25°23′449″ N; latitude 181.7 m) and Changsheng Village (114°58′1 418″ E, 25°23′2 010″ N; latitude 199.1 m) in Xinfeng County, Jiangxi Province. The navel orange trees were all ten years old, in their most productive period. The soil type was red soil. In each navel orange orchard, 15 sample spots were laid out evenly according to the size of the area. In order to ensure the representativeness of the sample, one well-grown navel orange tree was selected as a sample within a radius of about 10 m.

2.1.2 Soil samples. For each navel orange tree, 6 – 8 sampling points were arranged evenly beneath the canopy. In each sampling point, 2 kg of the top soil (0 – 20 cm) was collected in July, 2015. After removing miscellaneous materials such as dead branches, broken leaves and gravel, the soil collected from each navel orange tree was mixed together, placed in a clean cloth bag, and taken back into the lab. Among the soil samples collected from each navel orange orchard, the soil of every three samples was mixed as one set. That is, five soil samples were collected eventually from each orchard. The excess soil was discarded by quartering. The retaining soil was air-dried, ground, passed through 2-mm sieve, and stored in polyethylene zip-lock bags for use.

2.1.3 Plant samples. For Xinfeng navel orange, mid-April is the full-bloom period, post mid-November is the best period for fruit picking, and sampling is conducted 90 d after flowing. On July 20, September 20 and November 20, 2015, 8 – 10 fruits and more than 500 g of leaves were collected from each navel orange tree. From each of the four directions in the middle and upper part of the periphery of each canopy and in the middle and upper part of the fruiting branches, 1 – 2 fruits were sampled randomly. From each of the four directions in the middle and upper part of the periphery of each canopy and in the middle part of new shoots, 15 – 20 leaves were collected randomly. After sampling, all the samples were quickly put into a refrigerator at 4℃ and brought back to the laboratory. The grouping of plant samples were conducted as soil samples, *i.e.*, five sets of leaf and fruit samples were collected from each orchard. Leaf and fruit indicators were averaged for two orchards. After removing the main veins, the leaves were rinsed with tap water, rinsed with deionized water, dried at 110℃ for 30 min and 80℃ to constant weight, crushed, passed through 2-mm sieve, and stored in polyethylene zip-lock bags in success. The fruits were peeled, dried at 60℃ to constant weight, crushed, passed through 2-mm sieve, and stored in polyethylene zip-lock bags for use.

2.2 Methods

2.2.1 Determination methods. The contents of the four LREEs (lanthanum, La; cerium, Ce; praseodymium, Pr; neodymium, Nd) in the soil and plant samples were determined using the inductively coupled plasma-mass spectrometry (ICP-MS).

2.2.2 Digestion methods. The soil samples were digested using aqua-perchloric acid digestion method. A certain amount (0.50 g) of each soil sample was placed in a digestion tube, added with 5 mL of aqua regia with a varispenser, and stood overnight. In a digestion furnace, the sample was digested at 120℃ until it turned white. Then, the digestion tube was taken out, cooled, added with 4-mL perchloric acid and stood for 24 h. The digested sample was diluted to 25 mL and filtered through 0.45-μm water filter. The filtrate was collected for use.

The digestion of plant samples was modified with reference to the *National Food Safety Standard*: *Microwave Digestion Methods for the Determination of Rare Earth Elements in Plant Foods* (GB 5009.94-2012). A certain amount (0.30 g) of each leaf sample or fruit sample was placed in a high-pressure digestion tank, added with 5-mL superiorly pure HNO_3 with a varispenser, and stood overnight. Three repetitions were arranged for each sample. The sample was placed in a microwave accelerated reaction system for digestion. The microwave digestion parameters were shown in Table 1. After the tank was cooled and exhausted (by opening the lid), it was dried on a temperature-controlled hot plate at 130℃ to dispel acids until it was approximately dried. Then, the digestion tank was taken out and cooled. The digestion solution was transferred to a 25-mL volumetric flask. The digestion tank was washed with ultrapure water for 3 – 5 times, and the washings were combined with the digestion solution. Finally, the digestion solution was diluted to 25 mL and filtered through 0.45-μm water filter, and the filtrate was collected for use.

Table 1 Parameters of microwave digestion

Steps	Temperature // ℃	Heating time // min	Hold time // min
1	120	5	5
2	160	10	5
3	180	10	15

3 Results and analysis

3.1 Change in content of LREE in the soil of navel orange orchard As shown in Fig. 1, the changes of the soil La, Ce, Pr and Nd contents in the ten sets of sampling points in the two navel orange orchards varied greatly. The highest content of Ce was 297.00 mg/kg, and the lowest content of Pr was 10.30 mg/kg. The average contents of Ce, La, Nd and Pr in the soil were 226.50, 96.70, 82.54, and 22.14 mg/kg, respectively. The maximum content-minimum content differences of Ce, La, Nd and Pr were 170.00, 135.60, 91.20 and 29.40 mg/kg, respectively. The change multiples (maximum/minimum) of Ce, La, Nd and Pr contents in the orchard soil were 2.34, 4.36, 3.41, and 3.85, respectively. The analysis of variance (Table 2) showed that the differences in content among the four LREEs and the differences in the content of the same LREE among the 10 sets of soil samples were all significant ($P<0.01$). Among them, Ce showed the highest average content and the highest content in each set of soil samples ($P<0.01$), and Pr showed the lowest average content and the lowest content in each set of soil samples ($P<0.01$). It indicated that in the soil of the navel orange orchards, the contents of the four LREEs ranked as Ce > La > Nd > Pr, and the differences in the contents of the four LREEs were in the order as La > Pr > Nd > Ce.

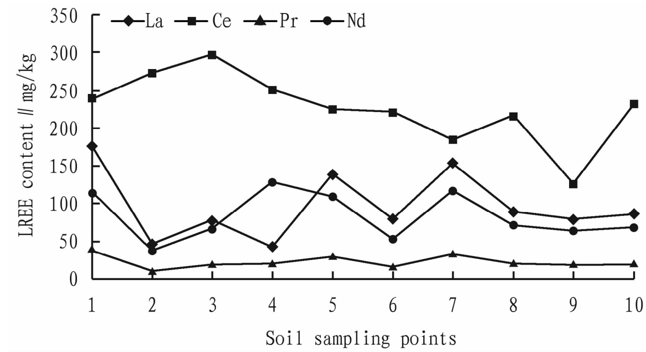

Fig. 1 Content of LREE in different sampling points of navel orange orchard

Table 2 Variance analysis of soil LREE content in navel orange orchard

Source of variation	Sum of squares				Degrees of freedom	Mean square				*F* value				*P* value
	La	Ce	Pr	Nd		La	Ce	Pr	Nd	La	Ce	Pr	Nd	
Different treatments	143.73	73.23	24.11	82.35	9	15.97	8.14	2.68	9.15	84.58	19.68	10.16	16.88	0.000 1
Within treatment	3.78	8.27	5.27	10.84	20	0.19	0.41	0.26	0.54					
Total variation	147.51	81.50	29.39	93.19	29									

3.2 Dynamics of contents of LREE in leaves and fruit of navel orange The dynamic curves of contents of the four LREEs in navel orange leaves at different growth stages (Fig. 2) showed that on July 20, the contents of the four LREEs in navel orange leaves ranked as La > Ce > Nd > Pr, the content of La was significantly higher than those of the other three LREEs ($P<0.01$), there was no significant difference between the contents of Ce and Nd, and the content of Pr was the lowest; during July 20 – September 20, the contents of the four LREEs in navel orange leaves tended to increase; on September 20, the accumulation amounts of the four LREEs in navel orange leaves reached the highest, and the contents of La, Ce, Pr and Nd were increased by 34.65%, 44.42%, 45.42%, and 45.47%, respectively; and from September 20 to November 20, the contents of the four LREEs in navel orange leaves showed downward trends, the decrease in Ce content (30.69%) was significant, while the decreases in La (5.12%), Pr (5.09%) and Nd (3.15%) contents were slow. In different growth periods, the total change trend of LREE in navel orange leaves was first increase and then decrease. The average contents of La, Nd, Ce and Pr in navel orange leaves ranked as La > Nd > Ce > Pr. The obvious accumulation period of LREEs in navel orange leaves was before September 20.

The dynamic curves of contents of the four LREEs in navel orange fruit at different growth stages (Fig. 3) showed that the contents of the four LREEs in navel orange fruit declined gradually. On July 20, the contents of the four LREEs in navel orange fruit ranked as La > Ce > Nd > Pr, and the differences in the contents of the four LREEs reached a significant level ($P<0.01$) in the early stage of the measurement; from July 20 to September 20, the contents of Pr, La, Ce and Nd in navel orange fruit reduced by 3.87%, 50.78%, 63.42%, and 40.16%, respectively; and from September 20 to November 20, the decline in LREE content was slow, and the contents of La, Ce, Pr and Nd declined by 25.67%, 12.65%, 22.50%, and 35.96%, respectively. During the growth period, the contents of La, Ce, Pr and Nd in navel orange fruit were reduced by 63.42%, 68.05%, 25.50%, and 61.68%, respectively. The migration rate of LREE in navel orange fruit was higher before September 20. The maximum content of LREE ($\leqslant 0.524$ mg/kg) in navel orange fruit was compared with the contaminant limit ($\leqslant 0.70$ mg/kg) in food (GB 2762-2012), and it could be concluded that the navel orange fruit met the requirements of green food.

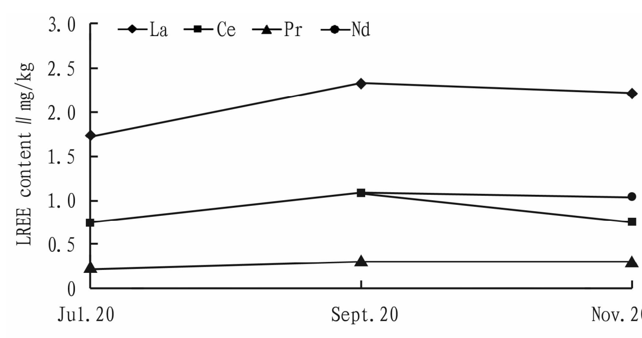

Fig. 2 Dynamic of LREE content in navel orange leaf

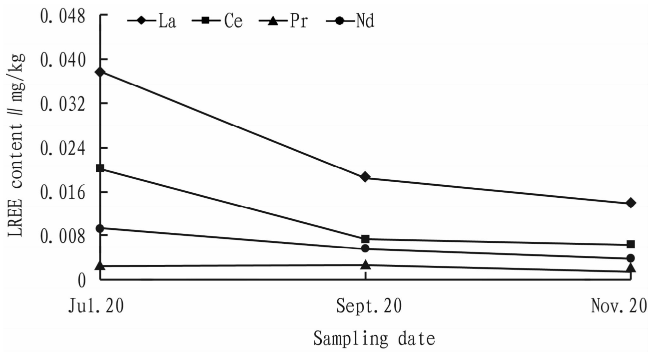

Fig. 3 Dynamic of LREE content in navel orange fruit

3.3 Content correlation analysis of LREE between orchard soil, leaves and fruit of navel orange Due to the influence of biological characteristics and the growing environment, the accumulation ratio of various rare earth elements in different fruit trees must have its inherent regularity[23]. There were clear liner positive correlations between LREEs in soil and leaves, soil and fruit, and leaves and fruit. Among the four LREEs, the correlation degree of Ce content between soil and leaves was highest, followed by that of La, and the correlation degrees of Pr and Nd were low (Fig. 4); between soil and fruit, the correlation degrees of the four LREEs were in the order as Pr > La > Ce > Nd (Fig. 5);

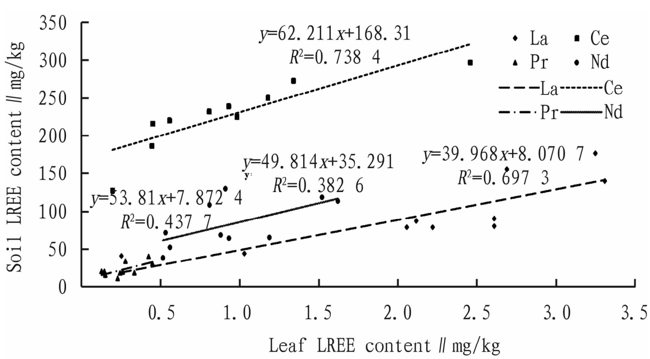

Fig. 4 Correlation analysis of LREE content between orchard soil and navel orange leaves

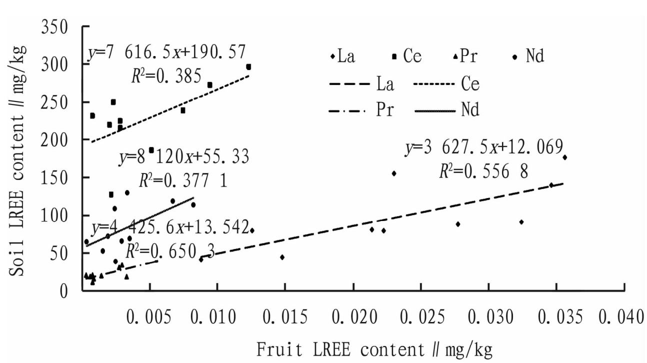

Fig. 5 Correlation analysis of LREE content between orchard soil and navel orange fruit

and the contents of La, Nd and Ce in navel orange leaves were all moderately correlated with those in navel orange fruit, and the content of Pr in navel orange leaves was weakly correlated with that in navel orange fruit (Fig. 6). The correlations of La content between soil and leaves, soil and fruit, and leaves and fruit, as well as the correlations of Ce content between soil and leaves, and leaves and fruit were moderate. Therefore, it was speculated that the accumulation amount of LREE in navel orange leaves and fruit was affected by LREE types; La might be the main LREE affecting the quality of navel orange, followed by Ce; and the correlation of LREE content between navel orange fruit and leaves was the highest.

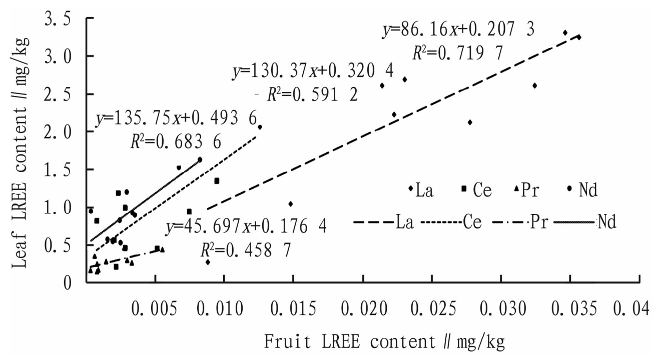

Fig. 6 Correlation analysis of LREE content between navel orange leaves and fruit

4 Conclusions and discussions

The impact of rare earth elements on plant growth and crop yield was studied earliest. In 1917, Chien and Ostenhout first reported the effect of rare earth element Ce on the physiology of spirogyra plants[24]. Scientists from the former Soviet Union first discovered in 1933 that La promotes wheat growth, while Ce inhibits wheat growth[25]. China is the earliest country in the world to apply rare earth fertilizers. Nongle and Changle are two commonly used mixed rare earth fertilizers. They have been widely used in rice, wheat, maize, rapeseed, sorghum, soybean, tomato, cucumber, Chinese cabbage, cress, pepper, *etc.*, more than 100 kinds of crops. They can improve crop quality and increase production by 5% – 15%[26–29]. The research on the influence mechanism of rare earth elements on the physiobiochemistry, photosynthesis, hormone metabolism, enzyme activity and stress resistance of plants are in-depth. Although rare earth elements have also been widely used in fruit trees such as apple, pear, jujube, hawthorn, grape, chestnut, peach, walnut, apricot, persimmon, plum, kiwifruit and citrus fruit and remarkable economic benefits have been achieved[30], the research focus on the effects of rare earth elements on fruit tree growth, fruit yield and fruit physiological indicators. The intrinsic mechanism of the migration of rare earth elements in fruit trees and the effect on fruit quality is not clear. Therefore, this study explored the correlations between rare earth elements in the soil navel orange tree system in Gannan navel orange orchards to facilitate the further study on the correlation between soil rare earth elements and navel orange quality in Gannan and improve the yield and quality of navel orange.

Fruit trees have a certain selectivity for the absorption of rare earth elements. Qi Guoliang *et al.*[31] determined the content of rare earth elements in soil and loquat fruit in six main producing areas of Ningxia and found that the total content of LREEs was higher than that of HREEs. This was consistent with the result of total rare earths in the soil and fruit of apple pear determined by

Shang Yuan *et al*[32]. Zhuang Maoqiang *et al.*[33] compared the contents of LREEs and HREEs in different fruits in rare earth mining areas and achieved similar conclusions. Zhong Linsheng[7] studied the distribution characteristics of rare earth elements in soils of Gannan navel orange orchards. It was found that the total amount of rare earth elements in the soil of navel orange orchard was higher than the average content of rare earth elements in the soil of mainland China (186.80 mg/g); the average content of LREE was 10.5 times that of HREE and dominated the total rare earth content; and the content of Ce accounted for 53.91% of the total rare earth content. Therefore, it was speculated that the contribution of LREEs in orchard soil to fruit quality was greater than that of HREEs. The correlations of LREEs between navel orange orchard soil and navel orange were studied in this paper. This lays a foundation for elucidating the mechanism of LREEs affecting the quality of navel orange and has guiding significance for the agricultural production of navel orange. The results showed that in the navel orange orchard soil, navel orange leaves and navel orange fruit, the contents of the four LREEs ranked as Ce > La > Nd > Pr, La > Nd > Ce > Pr, and La > Ce > Pr > Nd, respectively. It suggests that although the content of LREE in navel orange leaves and fruit is affected by the total amount of LREEs in the soil, there are certain differences in the accumulation of LREE among different organs of navel orange, and fruit trees are selective for absorption of LREEs.

Rare earth elements are not essential elements for the growth of fruit trees. Rare earth elements have certain mobility in the soil-fruit tree system. The accumulation of rare earth elements in different parts of fruit trees is closely related to the content of rare earth elements in soil. Liu Pinghui *et al.*[6] found that the contents of rare earth elements Ce (29.36 μg/g), La (16.92 μg/g) and Nd (8.62 μg/g) in the Nanfeng orange-growing soil in Jiangxi were highest, and their contents in the Nanfeng orange fruit were also higher (0.63, 0.52, and 0.12 ng/g), indicating that the absorption of rare earth elements in Nanfeng orange showed a positive correlation with the content of rare earths in soil. The results of this study showed that there were significant positive correlations in the contents of the four LREEs between navel orange orchard soil and navel orange leaves and fruit in Xinfeng County of Gannan area. During the process of evolution and migration from soil to leaves to fruit, the content of LREE gradually decreased. The differences in leaf LREE content were great, while the differences in fruit LREE content were small. This was consistent with the finding of Wang Zhenli *et al*[34]. So far, no in-depth study on the accumulation of rare earth elements in different parts of fruit trees has been conducted. Most studies[35-36] showed that the accumulation of rare earth elements in different organs of crops basically follows the order as roots > stems > leaves > flowers > fruit > seeds. It is inferred that the larger the migration distance of rare earth elements from the soil is, the lower the content of rare earth elements in fruit tree is. In spite of the high content of rare earths in the soil or navel orange roots and leaves, the accumulation of rare earths in the pulp as a terminal organ of navel orange may be very low due to the role of soil-plant barrier and the selective absorption and controlled accumulation of navel orange plant itself.

Studies have confirmed that moderate amounts of rare earth elements not only promote plant growth[37-38], and are also beneficial to human health. However, when exceeding the critical values, the growth of plants will be inhibited or even poisoned, and the health of human beings will be threatened[39-40]. Xie Wenlong *et al.*[41] studied the effects of contents of 26 mineral elements in the leaves of Newhall navel orange on the quality of fruit, and recommended appropriate accumulations for N, P, K, Ca, Mg, Cu, Zn, Fe, Mn, B, Mo, S, Cl and three kinds of limited elements Ni, Cd, La in navel orange leaves. The content of La is better not higher than 12 mg/kg, and the limits for other rare earth elements have not been reported. Therefore, the remobilization characteristics of different LREEs in the soil-navel orange tree system, and the limit values of LREEs accumulated during the benign development of the navel orange system remain to be further studied.

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