

Study on Screening of Vegetable Varieties with Low Accumulation of Rare Earth Cerium and its Accumulation Characteristics

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Abstract In this study, 17 kinds of vegetables were selected to make a contrast test in a nutrient solution cultured under the condition of 50 mg/L Ce pollution. *Lactuca Sativa L.*, a low enrichment and normal growth, was selected by soil experiment to analyze the accumulation of Ce and its physiological and biochemical effects. The results of the experiments are shown as follows: (1) 17 kinds of vegetables grew in good condition under the same cerium concentration hydroponics. The results showed that the content of Ce was the highest in the edible part of Brassica Juncea, up to 98.21 mg/kg, while cerium accumulation in the edible part of *Lactuca Sativa L.* was the lowest, only 6.64 mg/kg. From the results we can identify that *Lactuca Sativa L.* belonged to the low accumulative vegetable. (2) Total and available cerium in rhizosphere soil of *Lactuca Sativa L.* showed a highly significant positive correlation ($P < 0.01$), and cerium accumulation in edible parts of vegetables is significantly positive correlated ($P < 0.05$) with the cerium content in soil. (3) With the increase of cerium content in soil, *Lactuca sativa L.* as low-accumulation vegetable varieties, its antioxidant enzymes (SOD, POD, CAT) activity, proline content in plants increased at first and then decreased, and the content of MDA manifests a rising trend in general. This research has significant meaning for ensuring vegetable food safety in polluted areas dealing with especially in rare earth mining areas.

China is the most abundant country of rare earth resources in the world. According to the "China rare earth conditions and policy" 2012 white paper issued by the State Council Information Office, China has 11.89 million tons of rare earth reserves, which occupies 23% of the world's total reserves^[1], and also meets more than 90% of the world's and the extensive use of rare earth fertilizer. Recently, due to improper exploitation and smelting of rare earth elements in the soil, leading to the increasing containing of rare the market demand, rare earth pollution has become increasingly serious, the accumulation of rare earth elements in vegetables also increased^[2-3].

Chen Zuyi^[4], *etal.* reported that the vegetables (*arrowhead, water chestnut and water celery*) have strong enrichment capacity of the rare earth elements promethium (147Pm), the edible part of the three vegetables have a 147Pm absorption coefficient of 1.29, 2.09 and 1.55 respectively. Li Xiaofei^[5] *et al.* reported that in Changting County rare earth mining area of Fujian Province rare earth elements in *taro and spinach* is respectively 3.68 and 0.92 mg/kg, which exceeds the

vegetable health standard limit (0.7 mg/kg). In addition, Lianget *al*^[6] reported that water and soil environment contains more rare earth in China's rare earth mine district, resulting in the vegetables' rare earth content is about 10-20 times higher than the limits of national food. Although the effect of rare earth elements on human and animal's health and safety has not been fully confirmed yet^[7], how to effectively reduce the enrichment and accumulation of rare earths in crops and to ensure the safety of agricultural production and the ecological health of residents has become one of the most popular research topics in the current environmental science.

Some plants can grow naturally on heavily polluted soil, especially the plants which have low content of heavy metals elements in the upper part and these plants are called "rejection plants"^[8]. Based on this characteristic, as long as the suitable vegetables is found, the quality and safety products can be guaranteed even the vegetables grow in slightly polluted soil or the content of heavy metal is near the threshold, which is of great importance to guide the regional vegetable production. Extensive researches have studied on the effect of rare earth on crops, but most of them are mainly focused on lanthanum instead of Ce. Ce has the most abundance among rare earth elements, and it is also the main elements in rare earth elements. Moreover, most experiments only concern about the effect of rare earth on vegetable growth and find a range of promotion or inhibition. There are few reports on the mechanisms on differences of accumulation among species. In this paper, the absorption and accumulation of rare earth elements in different vegetables were examined by pot experiment, and the vegetable with low accumulation of rare earth elements were screened out. The mechanism of Ce accumulation in vegetable accumulation is discussed, which is of great significance for scientific and safe utilization of contaminated soil. At the same time, it provides a scientific evidence for the vegetables' safety in polluted areas, especially in the rare earth mining areas.

Materials and methods

Screening experiment

The experiment used Hoagland nutrient solution method. The method is as follows. Potted the similar growth status of plants with pH between 6 and 6.5, aerated the plant for 8 hours a day, replaced the nutrient solution every 3 days, and maintained continuous ventilation. Added Ce^{3+} to the nutrient solution ($LaCl_3 \cdot 7H_2O$) after the seedlings adapted to the nutrient solution in the basin environment, the mass concentration was set to 50mg/L (Ce^{3+}), repeated 3 times per process. After the samples were cultured for 20d under natural conditions, average value is taken for statistical analysis used for growth record and physiological parameters.

Concentration gradient test

The plants used for experiments were *Lactuca Sativa* L. Set Ce^{3+} to 6 levels (to Ce^{3+}): 100, 300, 600, 900, 1200, 1500 mg/kg and joined in the form of $LaCl_3 \cdot 7H_2O$, repeated 3 times per process.

Sample analysis and data processing

By dry ashing method, accurately weighed 0.500g samples to the porcelain crucible. Carbonized at low temperature for 1~2 hours with electric heating plate (for heating evenly, put in 2mL 1% HNO_3). Placed the porcelain crucible in the muffle furnace, heated to 200 °C for 30 min (until no smoke). Then waited 0.5 hours each 100 °C temperature increased. Gradually increased to 600 degrees, ashing can be finished about 5~6 hours later. Took out the sample and cooled to room temperature. Then added 5mL 10% HNO_3 to the porcelain crucible, placed on the electric heating plate for extraction at low temperature, until the dissolution of residual volume in crucible was about

0.5 ml. Added 3mL 1% HNO_3 to dissolve the residue and transferred to the 50mL volumetric flask. And then fixed to the calibration line and reserved.

Cedetection: Inductively coupled plasma emission spectrometer method (ICAP6000 Thermo, Scientific), Ultraviolet visible spectrophotometry method (723, Shanghai Jinghua Scientific Instrument Co. Ltd.); SOD activity: NBT photochemical reduction method; CAT activity: potassium permanganate titration method^[9].

Result analysis

Contrast test

In general, the Enrichment characteristics of a plant for an element are characterized by enrichment coefficients and transmission coefficients. Enrichment coefficient represents the degree of difficulty of element migration between medium and plant to a certain extent, and the accumulation trend of reaction medium in organism^[10]. Transmission coefficient is an important parameter to describe the uptake and transport of heavy metals by plants grown in heavy metal polluted soil, and the transmission capacity of plants to medium. As shown in table 1, the absorption levels of rare earth Ce in different vegetables are quite different. The Ce accumulation ability in edible part and root of *Lactuca Sativa* L. are relatively low, only 6.64mg/kg and 41.96 mg/kg, respectively. The enrichment coefficient and transfer coefficient are only 0.13 and 0.16, respectively. While other vegetables have relatively strong accumulation ability of Ce, the edible part of mustard can reach 98.21mg/kg.

Table 1 Concentration of Ce in 17 vegetables of edible parts

Number	Vegetable species	Classification	CeContent (mg/kg)	Enrichment coefficient	Transfer coefficient	Remarks
1	<i>Lpomoa aquatica</i>	Non edible part	199.95	1.08	0.27	The stems and leaves are edible, the roots are not edible
		Edible part	53.90			
2	<i>Spinacia oleracea</i>	Non edible part	357.21	1.52	0.21	The stems and leaves are edible, the roots are not edible
		Edible part	76.16			
3	<i>Amaranthus tricolor L.</i>	Non edible part	735.27	0.66	0.04	The stems and leaves are edible, the roots are not edible
		Edible part	32.91			
4	<i>Hedyotis hedyotideae</i>	Non edible part	37.06	0.33	0.44	The stems and leaves are edible, the roots are not edible
		Edible part	16.40			
5	<i>Brassica compestris L.</i>	Non edible part	935.62	1.65	0.09	The stems and leaves are edible, the roots are not edible
		Edible part	82.55			
6	<i>Allium fistulosum</i>	Non edible part	87.33	0.38	0.22	The stems and leaves are edible, the roots are not edible
		Edible part	19.17			
7	<i>Brassica juncea</i>	Non edible part	643.98	1.96	0.15	The stems and leaves are edible, the roots are not edible
		Edible part	98.21			
8	<i>Daucus carota</i>	Non edible part	27.81	0.50	0.89	The roots are not edible
		Edible part	24.78			
9	<i>Brassica campestris L.</i>	Non edible part	699.12	0.82	0.06	The stems and leaves are edible, the roots are not edible
		Edible part	41.09			
10	<i>Chrysanthemum coronarium</i>	Non edible part	107.48	0.36	0.17	The stems and leaves are edible, the roots are not edible
		Edible part	17.76			
11	<i>Brassicachinensis L.</i>	Non edible part	489.27	0.94	0.10	The stems and leaves are edible, the roots are not edible
		Edible part	47.05			
12	<i>Lactuca sativa L.</i>	Non edible part	41.96	0.13	0.16	The stems and leaves are edible, the roots are not edible
		Edible part	6.64			
13	<i>Coriandrum sativum</i>	Non edible part	162.63	0.31	0.09	The stems and leaves are edible, the roots are not edible
		Edible part	15.34			
14	<i>AtuberosumRottl.</i>	Non edible part	264.45	0.35	0.07	The stems and leaves are edible, the roots are not edible
		Edible part	17.47			
15	<i>Nasturtium officinale R. Br.</i>	Non edible part	539.36	1.38	0.13	The stems and leaves are edible, the roots are not edible
		Edible part	69.31			
16	<i>Brassica pekinensis</i>	Non edible part	210.87	0.79	0.19	The stems and leaves are edible, the roots are not edible
		Edible part	39.57			
17	<i>Capsella bursa-pastoris</i>	Non edible part	196.51	0.83	0.21	The stems and leaves are edible, the roots are not edible
		Edible part	41.55			

The ability of absorption and accumulation of Ce in *Lactuca Sativa L.*

Relationship between total Ce content and available content in rhizosphere soil

The data show that the absorption and utilization of heavy metals of plants mainly depend on the available content of plants. The total amount of heavy metals in soil and the available

components can transform with each other, and a dynamic balance between them can be reached. The total amount of rare earth Ce has great influence on the soil effective state. Figure 1 is the changes of the available content and the total amount of Ce in *Lactuca Sativa L.* rhizosphere soil.

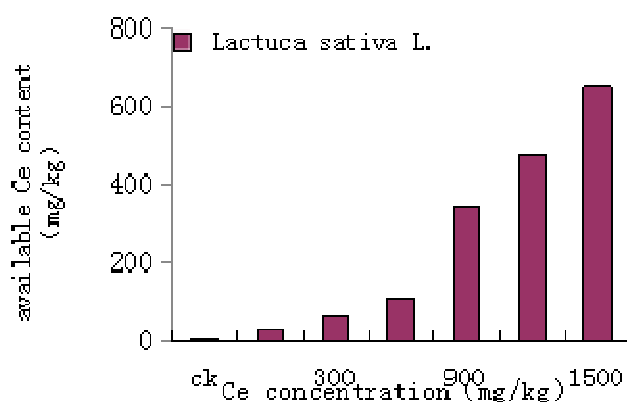


Fig1 Relationship between total Ce and available Ce content in soil

As shown in Figure 1, with the total amount of rare earth Ce in soil increased, the corresponding available Ce content also increased significantly. According to the linear regression equation of rhizosphere soil available Ce and total Ce, the correlation coefficient of *Lactuca Sativa L.* root rhizosphere soil available Ce and total volume is 0.978 which is a highly significant positive correlation ($P \leq 0.01$).

The ability of absorption and accumulation of Ce in *Lactuca Sativa L.*

As shown in Figure 2, roots and edible part of the *Lactuca Sativa L.* for accumulated amount of Ce with overall increased content in soil, and the root is larger than the edible part. When the soil concentration of Ce is less than 1200mg/kg, the absorption amount of Ce in *Lactuca Sativa L.* roots and edible part increased gradually. The root for the cumulative Ce most reached 132.31 mg/kg, and the edible part for the cumulative Ce uped to 8.87 mg/kg. When the concentration of Ce was greater than 1200mg/kg, the absorption rate of Ce decreased significantly, which indicated that the absorption of Ce was better under the condition of low concentration. High concentration of Ce affected absorption of Ce, and absorbed Ce more concentrated in the roots, less transferred to the edible part.

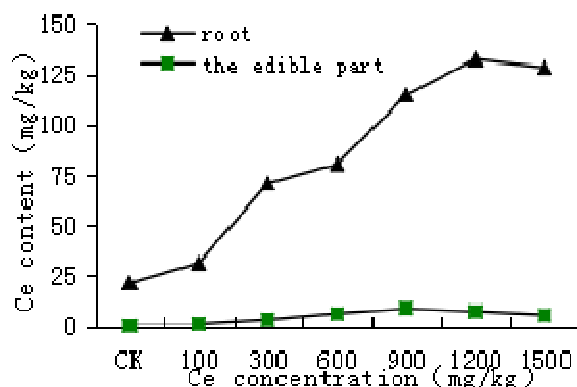


Fig.2 Concentration of Ce in Lactuca sativa L. under Ce treatments

Further analysis shows the correlation of soil and vegetables in total and available Ce contents of lettuce between the edible part of the content of Ce and Ce was significantly related to soil ($P < 0.05$), the edible part of mustard Ce content and soil available Ce content is not related. That

maybe because the high concentration of Ce inhibited the growth of vegetables, it has an effect on the transfer of Ce; may also be lettuce as low accumulated plants, which itself has an *in vitro* antibody, can effectively reduce the Ce transportation to the overground part .

Effects of Ce stress on antioxidant enzymes of lettuce *in vivo*

SOD, CAT and POD as an important enzyme in plant defense, which plays an important role in scavenging free radical. Under normal conditions, SOD, CAT, POD form a completely protective enzyme system, and make the protection mechanism from destruction. Thus, a dynamic balance of free radical production and removal is achieved within the plant, and the possibility of preventing plant cells from being injured is increasing. Therefore, changes in the activities of SOD, CAT and POD are often regarded as one of the tolerance indices for plants under heavy metal stress.

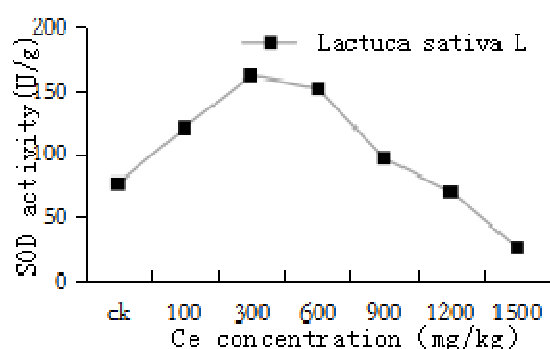


Fig.3 Effects of SOD in vegetables under Ce treatments

As shown in Figure 3, with the increases to the concentration of Ce, SOD activity in lettuce was firstly increased and then decreased. In lettuce, Ce concentration in the range of 0-300 mg/kg, SOD activity sustained growth until reached the maximum value of 152.1U/g at 300mg/kg. When concentration of Ce was higher than 300mg/kg, SOD activity was decreased, until the concentration less than control group. Experiments show the low concentration of Ce, because the rare earth as trace elements can promote plant growth and development, and there is no obvious side effect on plant growth. Superoxidedismutase activity maintained a relatively low level, but the concentration exceeded a certain range, the plants were persecuted, and stimulate the activity of superoxide dismutase. To enhance the ability to resist the damage of active oxygen plant, and reducing the damage of high concentration of Ce to plant, SOD activity was at a higher level, until the high concentration of Ce destroyed of the defense system.

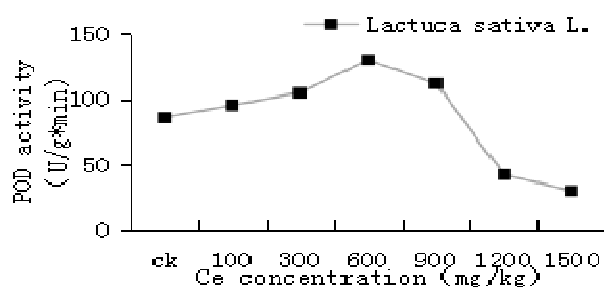


Fig.4 Effects of POD in vegetables under Ce treatments

Figure 4 shows the activity of POD in lettuce. With the increase of Ce concentration increased and then decreased, and reached its peak in the concentration of Ce at 600mg/kg, it was higher than control group 51.73%. With the increase of Ce concentration, the POD activity began to decline, and when the concentration of Ce is 1500mg/kg, the SOD activity was significantly lower than the control.

As shown in Figure 5, with the increase of Ce concentration, the activity of CAT increased firstly and then decreased. The concentration of Ce was 600mg/L, the CAT activity reached the maximum value of 202.61mg/(g·min·calls), 78.57% higher than the control, but the concentration of Ce in 1500mg/L, CAT activity was significantly lower than 52.56% of the control, the activity of CAT has been seriously inhibited, the high dose of Ce has destroyed the normal function of catalase.

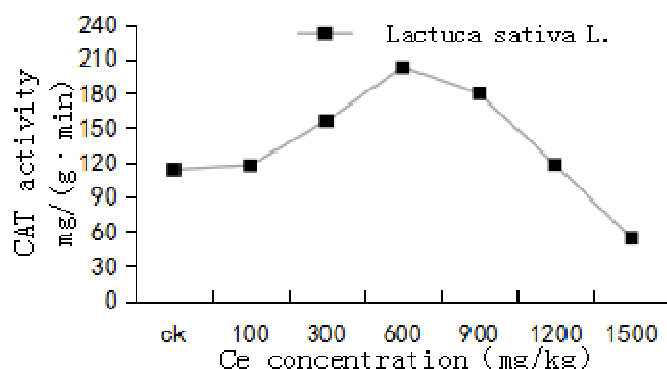


Fig.5 Effects of CAT in vegetables under Ce treatments

Effects of Ce stress on Proline and MDA in lettuce

Proline can be used not only as osmotic adjustment substances involved in the regulation, but also protect biological membranes, protein substances, scavenge active oxygen and other functions, and play a very important role in protecting cells from heavy metal stress^[11]. Therefore, free proline is often regarded as the main parameter to evaluate plant resistance to external stresses.

As shown in Figure 6, in the concentration range of Ce of 0~900 mg/kg, proline content in leaf lettuce show an upward trend, when the concentration of Ce is 900 mg/kg, the proline content reached the highest 363.81ug/g, this is the physiological limit of concentration; When the concentration of Ce was 900 ~1500 mg/kg, the proline content in lettuce decreased. The results showed that, with the increase of Ce concentration, in order to resist stress, the plant initiated physiological reaction to resist the harm of external environment, which resulting in a sharp rise in proline content. When the concentration of Ce further increased, the content of proline decreased rapidly, which indicated that the plant cells had been severely damaged and could not be recovered.

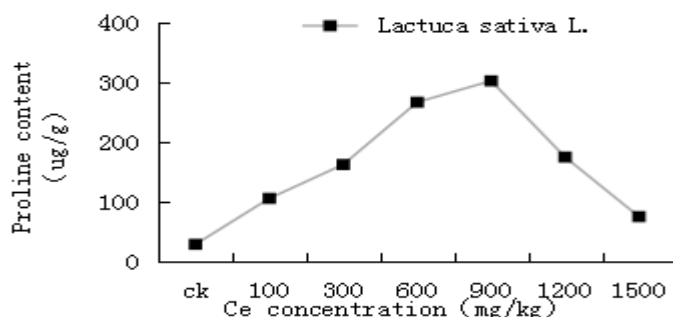


Fig.6 Effects of Proline content in vegetables under Ce treatments

Figure 7 shows the effect of Ce on MDA content of lettuce leaves. The content of MDA in leaves increased continuously with the increase of Ce concentration. When the concentration of Ce was 1500 mg/kg, it was significantly higher than the control group, but not yet reached its threshold. This indicated that, with the increase of Ce stress, the increase of MDA content aggravated the peroxidation of cell membrane lipid, resulting in non-recoverable damage to plants.

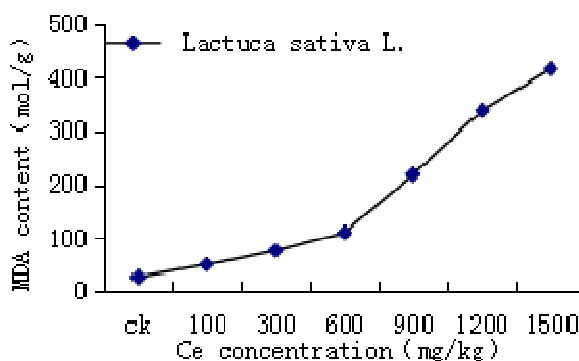


Fig. 7 Effects of MDA in vegetables under Ce treatments

Discussion

Vegetables are indispensable food to people's daily life, but the situation of rare earth pollution in the farmland is becoming more and more serious, and the situation that the vegetables are polluted by rare earth is not optimistic. It is very significant to figure out how to use contaminated soil reasonably. Screening low accumulation of heavy metals in crops and cultivating breeding genotypes of low accumulation crops is an effective method to reduce the amount of rare earth elements absorption and make vegetables harmless. However, the studies by now mainly focus on the crops especially in the differences of the accumulation of rare earth elements in profitable crops. The difference about absorption of rare earth among diverse vegetables is seldom studied.

It is found that rare earth metals have different forms in soil, and their content is affected by many factors, such as pH value, CEC and so on. And the available ion is the most easily absorbed form by plants^[12], which greatly affects the absorption and accumulation of metals. Meanwhile, the vegetation will also promote the transformations of rare earth metals into different forms when they restore rare earth elements in soil, from the original single state to a variety of forms coexisted. And the effective content changes a lot. This may be because both soil microorganisms and plant root exudates participate in the transformation of rare earth elements during the vegetation growth process^[13], which leads to the change to state more available for plant absorption.

In this study, edible portions of vegetables exhibited weak accumulation capacity for Ce, with only 6.64 mg/kg, and the root retention is the main approach of *L. sativa* accumulated Ce. It is generally believed that the retention effect of plant roots about rare earth metals may be a self-protective measures of plants in order to avoid excessive heavy rare earth permeate into stems and leaves to reduce the toxic effects of heavy metals^[14], may also be lettuce as low accumulation of vegetable plants, which itself has an vitro-antibody can effectively reduce Ce transportation to its ground. In addition, mustard's edible part of Ce accumulation can up to 98.21 mg/kg, which is higher than the edible parts of vegetables, and the Ce tolerance is also strong, with strong ability to adapt to the mustard may have a contact, so for as the mustard with high enrichment of rare earth elements is not suitable for planting vegetables and be used for food in a long period. Wang et al^[15] also found that the average content of rare earth elements in leafy green vegetables is relatively high.

This difference is very similar to vegetables absorb heavy metals. Therefore, the content of rare earth elements in vegetables not only depends on the type of vegetables, but also related to the growth media of edible vegetables, which dominate the absorption and transfer of rare earth elements.

Normally, SOD, POD and CAT can form an effective scavenging system of active oxygen radicals to maintain the dynamic balance of free radicals in plants. When plants subjected to external environmental coerced, it will destroy the dynamic balance. The effects of rare earth elements on plants are related to reactive oxygen species (ROS) in the body. This study shows that, with the increase of soil Ce content the SOD, POD, CAT and proline of lettuce leaves first increased and then decreased; The content of MDA is totally on the rise. The possible reason is the treatment of low concentration Ce, which plays a role in promoting the growth of vegetables, this moment the plants have a normal growth, so the ROS in plants do not increase, the leaf antioxidant defense system is not fully activated to remove the excess ROS, SOD, POD, CAT activity was only slightly increased, with the concentration increased, the activity of CAT, SOD and POD increased rapidly to remove excess ROS, and proline as active oxygen quencher to helping the antioxidant system remove ROS and increase membrane stability, which allowing cells or tissues to retain moisture. But the protective effect of the antioxidant defense system is limited, when Ce concentration continues to rise, the function of plant defense system in the body disorder, ROS scavenging ability is abated, excessive accumulation of cells' ROS, resulting in cell membrane lipid peroxidation increasing, this moment MDA content increased rapidly, to inhibit the activity of protective enzymes and reduce the content of antioxidant^[16].

Conclusion

(1) In this study, 17 vegetables grew well under the same Ce concentration gradient. The results showed that the edible part of mustard Ce enrichment was the highest, up to 98.21 mg/kg, and lettuce in the edible part of Ce accumulation was the lowest, only 6.64 mg/kg, and lettuce belongs to the low accumulation of vegetable varieties.

(2) Low concentration Ce treatment can promote the growth of plants, but when more than a certain concentration, they will inhibit the plants' grow. The study found that Ce total and available in Lettuce root rhizosphere soil showed significant positive correlation ($P < 0.01$), the accumulation of Ce in the edible part increased with the increase of Ce content in soil, and showed a significant correlation ($P < 0.05$).

(3) With the increase of soil Ce content, antioxidant enzymes (SOD, POD, CAT) activity in low Ce accumulation in vegetables lettuce, proline content showed an increase trend at first and then downward; the content of MDA is totally on the rise. On the whole, the toxic effects of Ce on Lettuce is more obvious than mustard, mustard is more tolerant to Ce.

This article studied on the 17 kinds of vegetables on the absorption and enrichment law of rare earth element cerium, and analyzed the reasons from physiological and biochemical indexes of lettuce low accumulation, our research results have reference value in the food safety of fresh vegetables and vegetable breeding etc.

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