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Migration of Selenium in Soil-rice-human System and Its Health Risk Assessment in Enshi

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Abstract In order to to provide scientific reference for the management and development of selenium resources in Enshi, four soil samples and four relevant rice samples were collected from four regions of Enshi, and then selenium content in the soil and rice samples were measured by ICP-MS. Afterwards, the occurrence forms of selenium in the soil samples were detected by continuous extraction. At last, the bioavailability of selenium in rice was studied by the *in vitro* gastrointestinal model. The results showed that selenium content in soil was 0.15 - 5.42 mg/kg in Enshi. The proportion of water-soluble selenium in soil was the lowest, ranging from 1.41% to 3.80%; the proportion of residual selenium was the highest, reaching 36.1% - 41.7%. Besides, selenium content in rice was 0.07 - 1.61 mg/kg, and the bioconcentration factor of selenium in rice was 0.22 - 0.48. The bioaccessibility of selenium in rice ranged from 45.7% to 56.4% in the stomach and from 58.4% to 68.5% in the small intestine. In addition, the daily intake of selenium per capita (PDI) in Yutangba, Changping, Shadi, and Taiyanghe was 490, 26, 132 and 57 μg/d, indicating that people in Yutangba had the risk of chronic selenium poisoning, and people's diet was rich in selenium in Shadi and Taiyanghe, while people's diet was deficient in selenium in Changping.
Key words Selenium, Soil, Rice, Bioaccessibility, Risk of selenium poisoning

1 Introduction

Selenium (Se) is a trace element necessary for the human body, but too much or less selenium is unfavourable to human health. Selenium deficiency in the human body can lead to Keshan disease, and excessive selenium will result in selenium poisoning such as alopecia and nail falling[1-2]. A moderate amount of selenium can resist oxidation, aging and cancer even. In the world, 2/3 of regions are deficient in selenium. It has been reported that diseases caused by selenium deficiency mainly appeared in more than 20 countries such as Finland, America and England. In China, more than 0.7 billion people's diet is deficient in selenium, and about 72% of counties and cities are deficient in selenium. Selenium deficiency has affected the development of human health and animal husbandry. Therefore, moderate selenium supplement is very important. Yutangba of Enshi in Hubei Province has the only selenium deposit mineralizing independently. Therefore, the creation and development of industry rich in selenium in Enshi is one of current and future strategies. However, the creation of industry rich in selenium must be based on basic scientific research. Hence, it is very urgent to study the migration, transformation and health risk of selenium in regions rich in selenium.

Research into the migration and transformation of selenium in Enshi has great significance to the cultivation of local crops. For instance, Xu *et al.*[3] studied the migration of selenium in soil-tobacco system in Enshi. Dai *et al.*[4] analyzed the migration of selenium in soil-tea system in Enshi. Rice is one of people's staple foods, so research on migration characteristics of selenium in rice has great significance to the assessment of people's health. At present, there has been no study on migration characteristics of selenium in rice-people system and assessment of its health risk. Scholars at home and abroad often adopted PDI (probable daily intake of Se for a human) index to evaluate the health risk of selenium, but researchers at home and abroad often calculated PDI value based on selenium content in food. However, selenium in food can not be absorbed by the human body fully, so PDI value calculated by the above method is increased. Therefore, PDI would be calculated based on the bioavailability of selenium for the first time in this study. Bioavailability refers to the proportion of food dissolved in gastrointestinal tracts of the human body after entering the digestive system of the human body[5]. Therefore, soil and rice samples containing different levels of selenium were collected from Enshi to study the migration of selenium in soil-rice-human, and the *in vitro* gastrointestinal model was used to study the bioaccessibility of selenium in rice and then assess the health risk of selenium in Enshi. The study aims to provide scientific reference for the development of industry in regions rich in selenium in China.

2 Materials and methods

2.1 General situation of the study area Enshi Tujia Miao Autonomous Prefecture is located in the southwest of Hubei Province and at the junction of Hubei Province, Chongqing City and Hunan Province. Here the people are simple and honest; good products from the earth are nature's treasures; there are beautiful mountains and clear waters. It is a western development area of

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China and is praised as "the capital of selenium in China". The only selenium deposit mineralizing independently in the world was found in Enshi. In Enshi, the outcrop area of carbonaceous rocks rich in selenium is about 850 km^2, and the thickness of seam is 3.6 m – 9.0 m; selenium content is the highest in the world, up to 8000 mg/kg.

2.2 Sample collection and treatment In the mature period of rice (on September 20, 2015), surface soil (0 cm – 20 cm) and corresponding rice grains (Yixiang 107, Keyou 21, Chuanfeng 6 and Guyou 102) were collected from Yutangba, Changping, Shadi and Taiyanghe of Enshi in Hubei Province. The soil was aired and crushed in the indoor shade, from which gravel, plant residue and other things were picked out. Afterwards, the soil was sieved by a sieve with 0.15 mm meshes and then was put in a polyethylene plastic bag. The rice grains were washed three times by running water and then by distilled water. They were aired at room temperature and was dried with a freeze drier. After their shells were removed, they were smashed, sieved by a sieve with 0.15 mm meshes, and stored in a polyethylene plastic bag.

2.3 Detection items and methods

2.3.1 Selenium content in soil and rice. Digestion of soil samples was conducted by the improved electricity plate digestion method of Williams *et al.*[6]. Firstly, 0.2 g of the aired, smashed and sieved sample was put in a digestion tube after the empty digestion tube was weighed, and then 10 mL of concentrated nitric acid and 2.5 mL of perchloric acid were added to the digestion tube. Afterwards, it was shaken well and stayed overnight. The next day it was heated on a low-temperature electric heating plate until white smoke could be seen, and then it was cooled when its volume was about 1 mL. Ultrapure water was added to it until its volume was 40 mL, and then it was weighed. The sample solution was filtered by 0.22 μm filter membrane and would be detected. Meanwhile, blank control was set, and each sample had three repetitions.

Digestion of rice samples was carried out by the microwave digestion method adopted by Wang Xin *et al.*[7]. Firstly, 0.2 g of the rice powder (that was sieved by a sieve with 0.15 mm meshes) was put in a microwave digestion tube, and then 2 mL of concentrated nitric acid and 2 mL of hydrogen peroxide were added to the tube. Afterwards, it stayed overnight and was digested by microwave. After the digestion was ended, it cooled down at room temperature. After the digestion tank was opened, the sample solution was put in a 50 mL centrifuge tube, and the digestion tank and its cover were washed with ultrapure water. The cleaning solutions were merged. Ultrapure water was added to it until its volume was 40 mL, and then it was weighed. The sample solution was filtered by 0.22 μm filter membrane and would be detected. Meanwhile, each sample had three repetitions, and blank control was set.

Selenium content in soil and rice was detected by ICP-MS (inductively coupled plasma mass spectrometry), and He collision pool mode was adopted by ICP-MS. The standard substances of soil constituent analysis were GSS-1 and GSS-3, while the standard substance of rice constituent analysis was GBW10010.

2.3.2 Occurrence forms of selenium in soil. Occurrence forms of selenium in soil were analyzed by the five-step continuous chemical extraction method of soil selenium[8], and the method was improved according to the samples of Enshi. To extract water-soluble selenium from the soil samples, 1.000 g of the soil was put in a 10 mL centrifuge tube, and then 10 mL of distilled water was added to the tube. It was covered and then was oscillated for 1 h at room temperature in a conical oscillator. Afterwards, it was centrifuged for 30 min at a speed of 4000 r/min, and supernatant was taken out and diluted with ultrapure water to certain volume. To extract exchangeable selenium, 10 mL of 0.1 mol/L KH_2PO_4 – K_2HPO_4 solution was added to the above centrifuge tube containing residue, and then it was oscillated for 2 h at room temperature. It was centrifuged for 30 min at a speed of 4000 r/min, and supernatant was taken out and diluted with ultrapure water to 10 mL. To extract selenium combined with iron and manganese oxides and carbonate, 10 mL of 3 mol/L HCl was added to the above centrifuge tube containing residue, and then it was heated for 50 min in water bath at 90 ℃. Intermittent oscillation was conducted, and then it was centrifuged for 30 min at a speed of 4000 r/min, and supernatant was taken out and diluted with ultrapure water to 500 mL. To extract selenium combined with organic matter, 10 mL of 0.1 mol/L $K_2S_2O_8$ was added to the above centrifuge tube containing residue, and then it was heated for 2 h in water bath at 90 ℃. Intermittent oscillation was conducted, and then it was centrifuged for 30 min at a speed of 4000 r/min, and supernatant was taken out and diluted with ultrapure water to 500 mL. To extract residual selenium, the residue was taken out, dried and ground again. Selenium content was measured by the method mentioned in **2.3.1**.

2.3.3 Bioconcentration factor of selenium. Bioconcentration factor of selenium in plants mainly reflect the ability of plants to absorb selenium. Bioconcentration factor of selenium in rice was the ratio of selenium content in rice to selenium content in soil.

2.3.4 Bioaccessibility of selenium in rice. Bioaccessibility of selenium in rice could be measured by the *in vitro* simulation digestion method[9]. To study the bioaccessibility of selenium in the stomach, 1.25 g of rice powder and 12.5 mL of gastric juice (6% pepsin, pH = 1.75) were put in a 50 mL serum bottle successively, and then the serum bottle was oscillated for 1 min – 2 min. Afterwards, it was sealed and oscillated for 3 h at 37 ℃ at a speed of 150 r/min. After being cooled, it was centrifuged for 20 min at a speed of 4000 r/min at 4 ℃. The sample solution was filtered with 0.45 μm filter membrane, sealed and stored at 0 ℃ – 4 ℃. Before being detected, it was diluted to 50 mL. Afterwards, 10 ml of the solution was diluted to 100 mL. To study the bioaccessibility of selenium in the small intestine, pH of the above solution was adjusted to 7.0 by using NH_4HCO_3. Afterwards, 10 mL of pancreatic juice (composed of 2% pancreatin and 0.2% bile) was added to the solution, and it was oscillated for 1 min. Other operations were shown above. Meanwhile, blank control was set, and each sample had three repetitions. Selenium content was measured by the method mentioned in **2.3.1**.

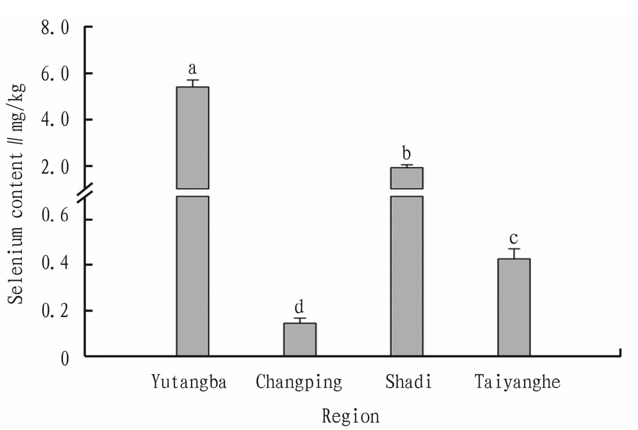
2.4 Assessment of health risk of selenium PDI index was used to assess the health risk of selenium, and its formula was $PDI = a \times b \times c / d$, where a is selenium content in rice; b is the bioaccessibility of selenium in rice, and the bioaccessibility of selenium in rice in the small intestine was adopted because selenium is mainly absorbed in the small intestine; c is the daily intake of rice per capita, and it was 217.8 g/d here[10]; d is the proportion of rice in all food, and it is 41.8% in Enshi[11].

2.5 Data processing Data statistics and correlation analysis were conducted by SPSS 19.0.

3 Results and analysis

3.1 Selenium content and its occurrence forms in soil in Enshi

3.1.1 Selenium content in soil. As shown in Fig. 1, selenium content in soil in Enshi ranged from 0.15 to 5.42 mg/kg, and its spatial distribution was uneven. That is, there were big differences between various regions in terms of selenium content in soil, and the order was shown as follows: Yutangba > Shadi > Taiyanghe > Changping. Selenium content in soil in Yutangba was the highest, up to 5.42 mg/kg, which was higher than the reference value of selenium content in soil in regions with selenium surplus in China[12]. Selenium content in soil in Changping was the lowest, only 0.15 mg/kg. According to selenium content in soil, soil can be divided three types, including soil rich in selenium (> 0.4 mg/kg), soil containing moderate amounts of selenium (0.2 – 0.4 mg/kg), and soil deficient in selenium (0.1 – 0.2 mg/kg)[13]. Therefore, soil in Yutangba, Shadi and Taiyanghe was rich in selenium, while soil in Changping was deficient in selenium.



Note: Various lowercase letters means there were significant differences between various regions in terms of selenium content in soil ($P < 0.05$). The same as below.

Fig. 1 Selenium content in soil in different regions of Enshi

3.1.2 Occurrence forms of selenium in soil. Besides selenium content in soil, selenium content in rice is closely related to occurrence forms of selenium in soil. Hence, it is necessary to study occurrence forms of selenium in soil. Seen from Fig. 2, the proportion of water-soluble selenium in soil was the lowest, ranging from 1.41% to 3.80%; the proportion of residual selenium was the highest, reaching 36.1% – 41.7%. Available selenium in soil refers to selenium that can be absorbed and utilized by plants, and its proportion in selenium in soil is approximately equal to the proportion of exchangeable selenium and water-soluble selenium. In Enshi, the proportion of available selenium in soil was 10.3% – 17.8%, which was low in most regions. A large proportion of selenium could not be utilized by plants, showing that the bioconcentration factor of selenium in soil in Enshi was low. In addition, there were small differences between various regions in terms of proportion of available selenium in soil, so selenium content in rice depended on selenium content in soil.

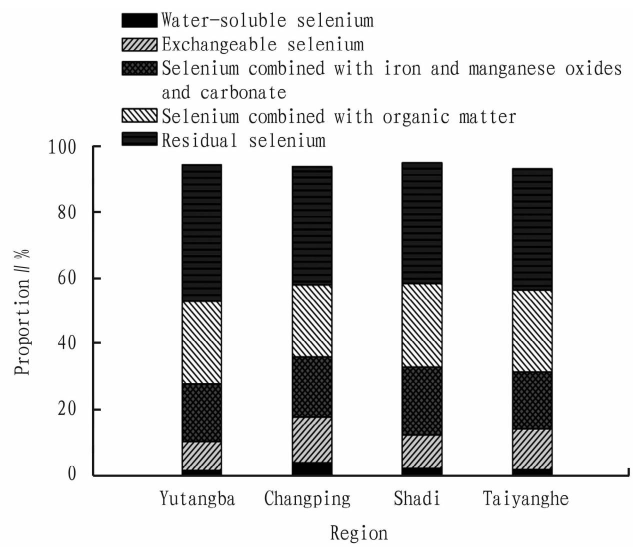


Fig. 2 Occurrence forms of selenium in soil in Enshi

3.2 Selenium content in rice and its bioconcentration factor in Enshi

3.2.1 Selenium content in rice. According to Fig. 3, selenium content in rice in Enshi was 0.07 mg/kg – 1.61 mg/kg. Among these varieties of rice, selenium content in Yixiang 107 in Yutangba was the highest, up to 1.61 mg/kg; selenium content in Keyou 21 in Changping was the lowest, only 0.07 mg/kg. Selenium content in Chuanfeng 6 in Shadi and in Guyou 102 in Taiyanghe was 0.43 and 0.17 mg/kg respectively. The distribution of selenium content in rice in Enshi was consistent with that in soil, and the order was shown as follows: Yutangba > Shadi > Taiyanghe > Changping. It is clearly seen that selenium content in rice was relatively high in regions where selenium content in soil was high. Selenium content in rice in Yutangba was close to selenium content (1.38 mg/kg) in a village suffering selenium poisoning[14] and was significantly higher than other regions of Enshi. Therefore, people who have eaten rice in Yutangba for a long time may be poisoned by selenium, which should be paid more attention to by local government. Selenium content in rice was low in Changping, so people who have eaten local rice for a long time may be deficient in selenium, and they should eat rice rich in selenium at the same time.

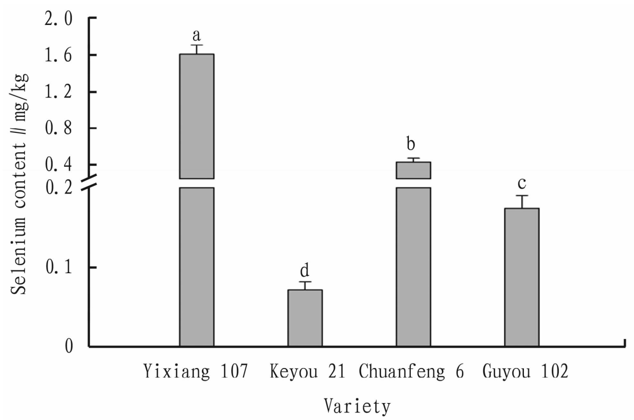


Fig. 3 Selenium content in different varieties of rice in various regions of Enshi

3.2.2 Bioconcentration factor of selenium in rice. Besides selenium content and its occurrence forms in soil, selenium content in rice is closely related to the physiological structure of rice. Bioconcentration factor of selenium in rice is an important indicator showing the ability of rice to absorb selenium. As shown in Fig. 4, there were significant differences between various varieties of rice in terms of bioconcentration factor of selenium, and it was 0.30, 0.48, 0.22 and 0.41 respectively. Among these varieties of rice, the ability of Keyou 21 to absorb selenium was the strongest, followed by Guyou 102, while the ability of Chuanfeng 6 to absorb selenium was the weakest. It shows that the variety of rice can not be neglected when selenium accumulation in rice is studied. It is suggested that suitable varieties of rice should be planted according to local conditions.

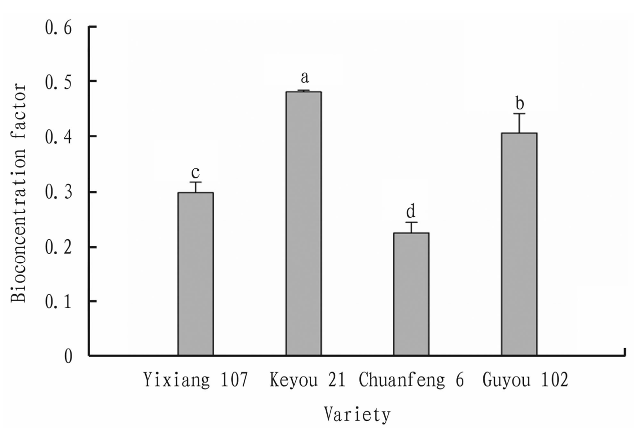
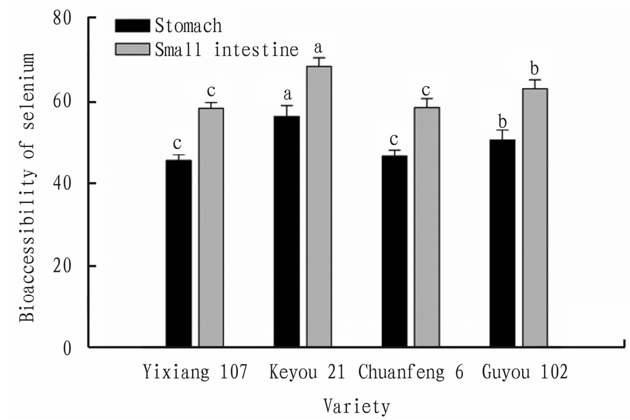


Fig. 4 Bioconcentration factor of selenium in different varieties of rice in Enshi

3.3 Bioaccessibility and assessment of health risk of selenium in rice in Enshi

3.3.1 Bioaccessibility of selenium in rice. After entering the human body, rice can be absorbed by gastrointestinal tracts. In this study, the *in vitro* gastrointestinal model was adopted to study the bioaccessibility of selenium in rice. Seen from Fig. 5, the bioaccessibility of selenium from various varieties of rice was different. It ranged from 45.7% to 56.4% in the stomach and from 58.4% to 68.5% in the small intestine, lower than 100%. The results reveal that rice can not be absorbed by the human body completely after entering the human body. In the small intestine and the stomach, the bioaccessibility of selenium in Keyou 21 in Changping was the highest, followed by Guyou 102 planted in Taiyanghe. The bioaccessibility of selenium in Chuanfeng 6 in Shadi and in Yixiang 107 in Yutangba was low, indicating that the effects of rice variety on the bioaccessibility of selenium in rice can not be neglected. The differences between various varieties of rice in terms of bioaccessibility of selenium in rice might be related to the content and proportion of each component in different varieties of rice.



Note: Various lowercase letters means there were significant differences between different varieties of rice in terms of bioaccessibility of selenium ($P < 0.05$).

Fig. 5 Bioaccessibility of selenium in different varieties of rice in Enshi

3.3.2 Assessment of health risk of selenium in rice. *PDI* index is often used to assess the health risk of selenium in people's diet. According to Fig. 6, *PDI* in Yutangba, Changping, Shadi and Taiyanghe was 490, 26, 132 and 57 μg/d respectively. According to the criteria for distinguishing the nutrient level of selenium in diet[15] and the latest allowable maximum dose of selenium specified by the World Health Organization (WHO)[16], as *PDI* is smaller than 40 μg/d, local people's diet is deficient in selenium; when PDI is 40 – 200 μg/d, the level of selenium in diet is normal; as PDI is 200 – 400 μg/d, diet is rich in selenium; when PDI is 400 – 800 μg/d, local people has the risk of chronic selenium poisoning. Therefore, people in Yutangba had the risk of

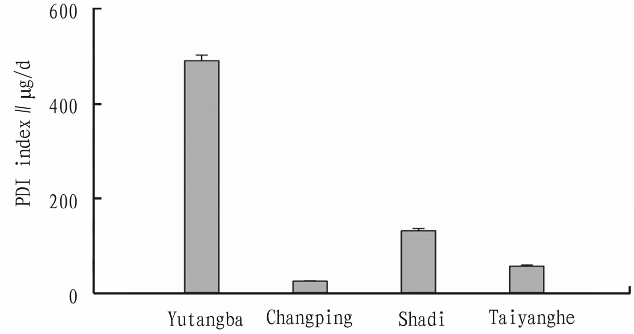


Fig. 6 *PDI* index in different regions of Enshi

chronic selenium poisoning, and people's diet was rich in selenium in Shadi and Taiyanghe, while people's diet was deficient in selenium in Changping.

4 Conclusions and discussion

In this study, the results showed that selenium content in soil in Yutangba of Enshi was 5.42 mg/kg, which was similar to the result of Zhu *et al.* [17] (4.75 mg/kg). In Yutangba, selenium content in rice was 1.61 mg/kg, which was slightly lower than the result of Sun *et al.* [8] (2.18 mg/kg - 8.44 mg/kg). The difference was related to the sampled varieties and sampling time.

Besides selenium content in soil, selenium content in rice is closely related to the occurrence forms of selenium in soil. In this study, the proportion of water-soluble selenium in soil was the minimum, ranging from 1.41% to 3.80%; the proportion of residual selenium was the maximum, reaching 36.1% - 41.7%. The results of Wei Shaowei *et al.* [19] showed that the proportion of water-soluble selenium in soil was the lowest, only 3.1%; the proportion of residual selenium was the highest, up to 42.0%. The bioconcentration factor of selenium in rice can also affect the migration of selenium in soil-rice system. The results showed that there were certain differences between various varieties of rice in terms of bioconcentration factor of selenium, so suitable varieties of rice should be planted according to local conditions.

In this study, PDI was calculated by using bioaccessibility of selenium. The results revealed that the bioaccessibility of selenium from various varieties of rice ranged from 45.7% to 56.4% in the stomach and from 58.4% to 68.5% in the small intestine, lower than 100%. It is clearly seen that rice can not be absorbed by the human body completely after entering the human body. After collecting rice samples from India, Jaiswal *et al.* [13] found that the bioaccessibility of selenium was 52% in the stomach and 65% in the small intestine. In addition, there were certain differences between various varieties of rice in terms of the bioaccessibility of selenium. In regions where selenium content in soil is low, people should eat rice with high bioaccessibility of selenium. In regions where selenium content in soil is high, people should eat rice with low bioaccessibility of selenium to avoid selenium poisoning.

In Enshi, PDI in Yutangba, Changping, Shadi and Taiyanghe was 490, 26, 132 and 57 μg/d respectively. In Yutangba, local people had the risk of chronic selenium poisoning, and local government should adopt measures to reduce the risk. People's diet was rich in selenium in Shadi and Taiyanghe. In Changping, people's diet was deficient in selenium, so local government should adopt measures to improve selenium deficiency in people's diet. By using selenium content in food, Huang *et al.* [20] calculated PDI index in Shadi of Enshi, only 550 μg/d; Qin *et al.* [11] calculated PDI index in a high selenium area of Enshi, up to 2144 μg/d. The above results were different from the result of this study. On the one hand, the difference was related to sampling time. On the other hand, PDI was calculated based on selenium content in food, while PDI was calculated based on the bioaccessibility of selenium. The human body can not absorb selenium in food, so the bioaccessibility of selenium was smaller than 100%. In this study, the maximum of bioaccessibility of selenium was 68.5%, so the method to calculate it in this study is relatively scientific and accurate.

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and Margalef species richness index showed that sampling points #2, 3# and 10# were α-moderate pollution, Shannon-Wiener diversity index and Margalef species richness index showed that the remaining samples were β-moderate pollution, Pielou evenness index showed 6# and 7# sampling points were o-mild pollution. The average value of three diversity indices indicated that the water of Small Xingkai Lake was β-moderate pollution.

6 Countermeasures for quality management of water resources in Small Xingkai Lake

6.1 Cause analysis of water pollution In the sampling points, the lowest values of the three indices were concentrated on sampling points 2#, 3# and 10# , which is due to the fact that return water of farmland irrigation was mainly concentrated on sampling points 2# and 3# , and agricultural pollution exerted a great influence on the water quality; sampling point 10# was situated in area with frequent human activities, domestic sewage significantly increased, the interference of human activities was great, and diversity index was relatively low. These indicated that the water quality of Small Xingkai Lake was greatly influenced by return water of farmland irrigation and human activities. The sampling points 7 # and 6 # were two sampling points having high index value, mainly because the upstream of sampling point 6# was wetland core area with wetland vegetation which has excellent purification effect on water body, while sampling point 7# was less influenced by human activities.

6.2 Countermeasures for quality management of water resources

6.2.1 Reducing the impact of human activities on the ecological environment. At present, there are residents and farmland in the core area of Small Xingkai Lake nature reserve. With the rapid economic and social development, the production and living of residents have posed a serious threat to the ecological and environmental safety of Small Xingkai Lake. In order to protect and repair the ecological environment of Small Xingkai Lake, it is necessary to carry out ecological resettlement for the residents in Xingkai Lake Nature Reserve, relocate all the residents in the core area, withdraw all the farmland, establish the new resettlement villages, establish auxiliary domestic rubbish, sewage, energy, and centralized heat supply system, and make compensation for houses, farmland, and other auxiliary facilities of villagers. In the process of resettlement, it is required to fully respect willingness and customs of farmers and villagers.

6.2.2 Developing ecological agriculture and controlling non-point source pollution. In order to reduce the application of nitrogen and phosphate fertilizer in farmland, we should take measures such as scientific soil testing and formula fertilization, increase the application of organic manure, and return straws to field. Besides, it is required to increase the utilization rate of highly effective and low toxicity pesticides and biological pesticides and gradually reduce the use of chemical pesticides. In the livestock and poultry breeding, it is recommended to promote large scale breeding, separate the manure and urine and separate rainwater and sewage, build small sewage treatment and manure treatment facilities, and return the treated manure and urine to the field. Finally, it is recommended to encourage and support joint operation of vegetable greenhouses and livestock and poultry breeding, establish " manure and urine-biogas-natural gas-greenhouse heating" integrated vegetable facilities, to provide vegetable with fertilizer, supply heat for greenhouse, and solve the problem of pollution of livestock and poultry manure. It is a green and pollution-free ecological development mode.

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