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Research Article

Influence of Land Treatment for Rural Sewage on Soil Environment and Flue-cured Tobacco Quality

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Abstract: In order to explore how the land treatment of rural sewage affects the soil environment and the flue-cured tobacco quality, the pretreated sewage collected from rural areas of south China were directly discharged to the experimental fields and the microbial number, enzymatic activities of soils with different distance away from the outlet and different depth were observed; besides, the sewage were diluted to different proportions and applied for the cultivations of flue-cured tobacco, afterwards, the flue-cured tobacco leaves at maturity were harvested for the quality evaluation and the Principle Component Analysis (*PCA*) method was adopted to select the best dilution proportion (treatment) based on the comprehensive analysis of the quality of tobacco leaves under different treatments. Results showed that: The sewage treatment had little effect on surface soil microbial number, but which significantly increased the number of bacteria, fungus and actinomyce in soils of 20~40 cm and 40~60 cm layer. At the same time, the sewage treatment significantly increased the soil enzymatic activity including the urease activity, catalase activity and polyphenol oxidase activity at all measuring points. Known from the analysis of flue-cured tobacco leaves, treatments with higher sewage proportion obviously increased the content of nicotine, total N and potassium in flue-cured tobacco leaves, but which decreased the content of reducing sugar. According to the calculations of *PCA* model, the quality sequence from excellent to inferior could be ordered as: T2>T1>T3>T4>CK, therefore, for flue-cured tobaccos, T2 was more conductive for equilibrium absorption of nutrients compared with other treatments.

Keywords: Principle component, quality, rural sewage, soil environment, tobacco

INTRODUCTION

The rapid growth of population, the indiscriminate discharge of modern industrial wastewater, the generating of municipal waste and the spraying of rural pesticide had already caused the shortage of freshwater resources, some of the polluted waters could not be used by mankind, according to statistics, there were more than 2000 kind of contaminants found in these polluted waters, leading to a serious environmental problem which threaten the human survival, therefore, the treatment of wastewaters had been a hot topic for researches from all over the world (Hamzeh *et al*., 2011; Varol, 2013; Zhou *et al*., 2013). In the south of China, sewage was an important component of rural wastes and the land treatment of rural sewage was a kind of wastewater treatment method with lower cost and smaller occupation space. However, rare studies were found in exploring the effect of land treatment for rural sewage on soil environment from the perspective of soil microbiology. The soil microorganisms and the soil enzyme almost participated in the entire chemical or biochemical process of soil ecosystem, the development of microorganisms and the activities of enzyme in soils reflected the soil health directly and which were important indexes to evaluate the soil quality (Castro *et al*., 2013; Gruyer *et al*., 2013; Jang *et al*., 2013).

On the other hand, rural sewage was also a type of available resource; the pretreated rural sewage could be used in the cultivation of crops on account of its abundant nutrients. Tobacco was a cash crop in China and was very important to China's national economy; hence lots of attention had been paid on the basic and apply studies of tobacco cultivation (Cao *et al*., 2013; Hou *et al*., 2013a, 2013b; Liu *et al*., 2009; Shao *et al*., 2012). In this study, the effects of land treatment for rural sewage on the soil microorganisms and the soil enzyme activities at different measuring points were observed and the sewage were also diluted to different

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concentrations, for exploring the effects of sewage irrigation with different proportions on flue-cured tobacco quality, in order to provide beneficial basis for rural sewage treatment and its agricultural recycling.

MATERIALS AND METHODS

Experimental site: The experiments were initiated from Match 2013 to October 2013 in Yancheng (latitude 33.38°, longitude 120.13°), Jiangsu province, China. The yearly mean temperature was about 13.7~14.5°C, maximum temperature was recorded as 37.2°C. The annual rainfalls of Yancheng was 785.2～1309.5 mm, with the number of rainy days about 96~113. Besides, Yancheng enjoys about 2280 h sunshine annually and spring, summer, autumn and winter occupied 25, 29, 24 and 22% respectively for the yearly sunshine hours.

Materials: The sewage was collected from the local rural areas and then transported to the experimental fields. Flue-cured tobacco K326 were chosen as plant materials, young plants are elaborately cultivated in made-in-order seedling trays and then they would be transplanted into the experiment fields when 6 expanded leaves appeared. After that, field management conforming to local habits was carried out in the first week.

Experimental design: The pretreated sewage was transported upwards by the underground perforated pipes, spreading slowly to the surrounding soils. 3 blocks (10 m×5 m) were divided for the experiments, the control blocks were accorded with the experimental blocks. In each block, five-point sampling method was adopted to collect the soils of 0~20 cm, 20~40 cm and 40~60 cm away from the water outlet, for studying the effects of land treatment for rural sewage on the microorganism number and soil enzymatic activities of the soils with different distance away from the sewage water outlet. On the other hand, at the location of 20~40 cm away from the outlet, the soils with 0~20 cm, 20~40 cm and 40~60 cm depth were collected for analysis. Waters used in the control blocks were running waters and the sampling method in control blocks kept the same with that in experimental blocks.

On the other hand, the pretreated sewage was also diluted to different concentrations for the flue-cured tobacco cultivation and the dilution proportion (mixing with running waters) was 1:1 (T1), 1:2 (T2), 1:3 (T3), 1:4 (T4) and CK (running water). Irrigation water amount of root-extending stage, vigorous stage and maturity stage accounted 30, 40, 30%, respectively for the total water consumption respectively, the waters were irrigated averagely into the soils every 7 days on the basis of the growth period division. Inorganic fertilizers special for flue-cured tobacco were applied according to the proportion of basal fertilizer: additional fertilizer = 7:3 (bought from Tobacco Institute of Science in Guizhou Province, N: P_2O_5: K_2O = 1:2:3) and latency time of the topdressing was about 26 days after transplanted.

Main tested index and methods: Microbial number: numbers of bacteria, actinomyces and fungi were measured by the plate count method (Soriano-Disla *et al*., 2014; Trötschel *et al*., 2013).

Soil enzymatic activities:

Urease: The urease activities were measured by the indophenol blue colorimetric method and the unit was expressed through the NH3-H amount (mg) produced by 1 g soil sample at 37°C after 24 h hydrolysis reaction.

Catalase: The catalase activities were measured by the $KMnO_4$ titration method and the unit was expressed through the volume (mL) of 0.02 mol/L $KMnO_4$ consumed by 1 g soil sample.

Polyphenol oxidase: The polyphenol oxidase activities were measured by the pyrogallol colorimetric method and the unit was expressed through the gallnut amount (mg) produced by 1 g soil sample at 30°C after 2 h reaction (Antonious, 2013; Panettieri *et al*., 2013).

Flue-cured tobacco quality: At maturity, 3 flue-cured tobacco plants were randomly chosen from each treatment, the lower leaves, middle leaves and the upper leaves of each plant were collected orderly, killed by 105°C high temperature, toasted to the constant weight and then mixed. Total N content was determined using the continuous flowing method (YC/T 161-2002); nicotine content was determined using the continuous flowing method (YC/T 160-2002); content of total sugar and reducing sugar were determined using the continuous flowing method (YC/T 159-2002) (Hou *et al*., 2012; Liu *et al*., 2009).

Data analysis: Data were analyzed using SPSS 17.0 software and the differences among treatments were analyzed based on Duncan's new multiple range test (Lawrence, 1984).

RESULTS AND DISCUSSION

Microbial number: In Fig. 1, there were no significant differences of microbial number between the sewage treatment and CK at different distances away from the outlet. On the whole, the bacteria number of sewage treatment was slightly higher than that of CK, while there was no obvious regularity found in the number of fungus and actinomyce, this probably because that the underground outlet system could hardly influence the surface soils. Figure 2 showed the microbial number of the soils with different depth, the bacteria number raised significantly with the increasing of soil depth, while no obvious differences of which were found in

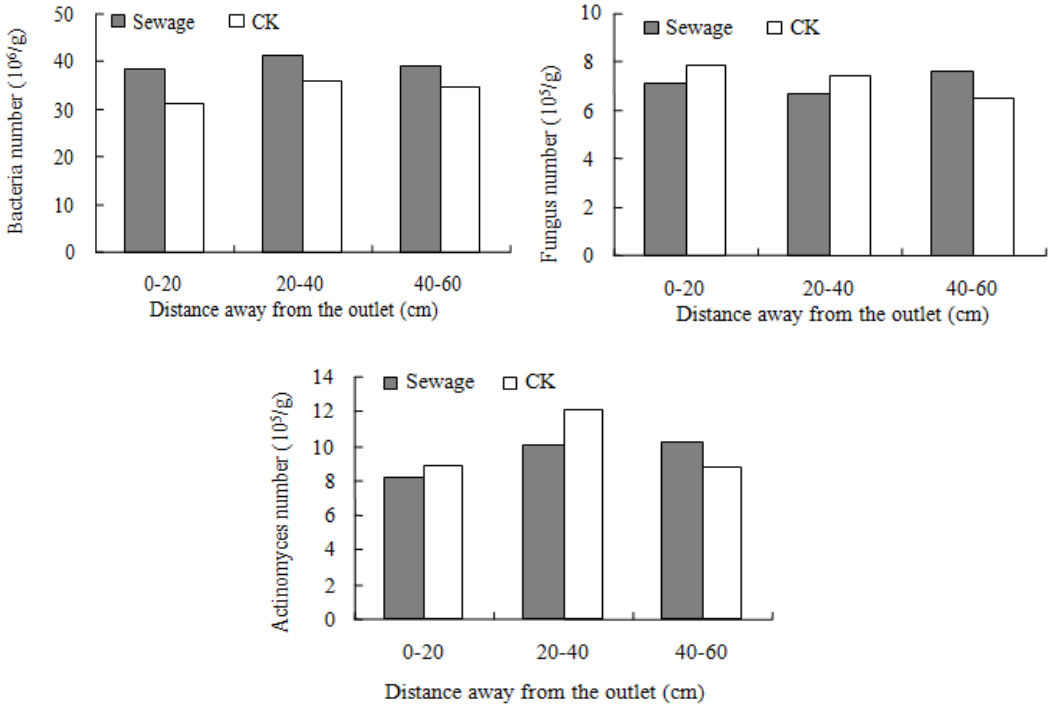

Fig. 1: The microbial number of soils with different distance away from the outlet

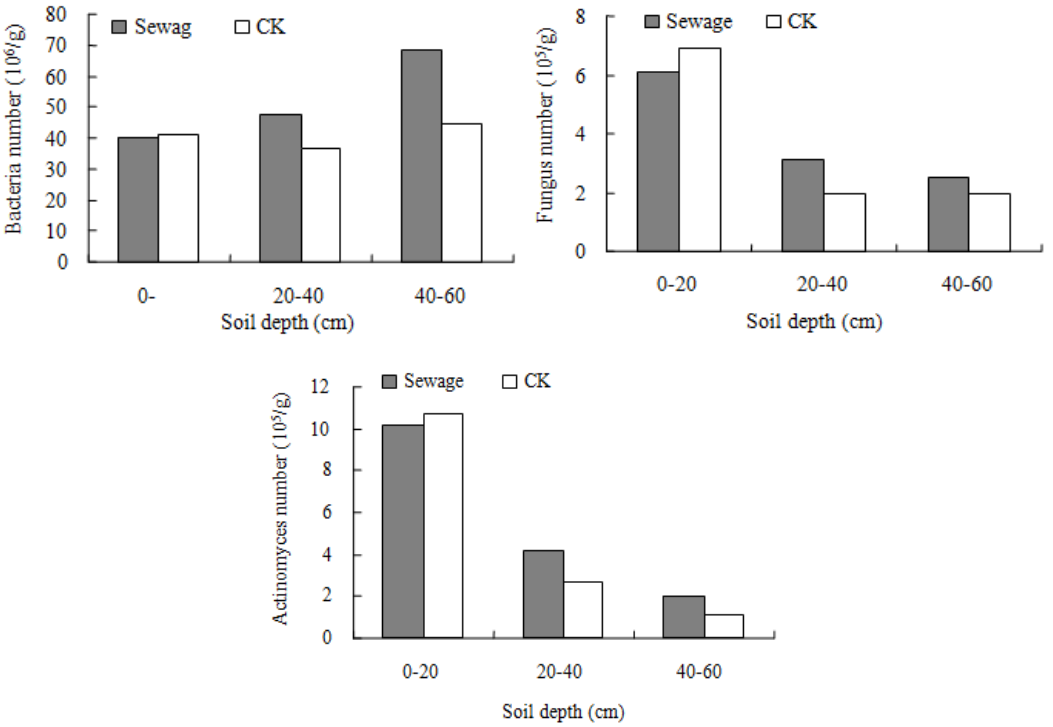

Fig. 2: The microbial number of soils with different soil depth

different soil layers of CK, indicating that the bacteria number had a positive relationship with the sewage amount; on the other hand, the number of fungus and actinomyce in 20~40 cm and 40~60 cm soil layer was obviously lower than that in 0~20 cm soil layer, moreover, in the same soil layer of 20~40 cm or 40~60 cm, the number of fungus and actinomyce of the sewage treatment was higher than that of CK, this result showed that the sewage had some effects on microbial number in deeper soil layer.

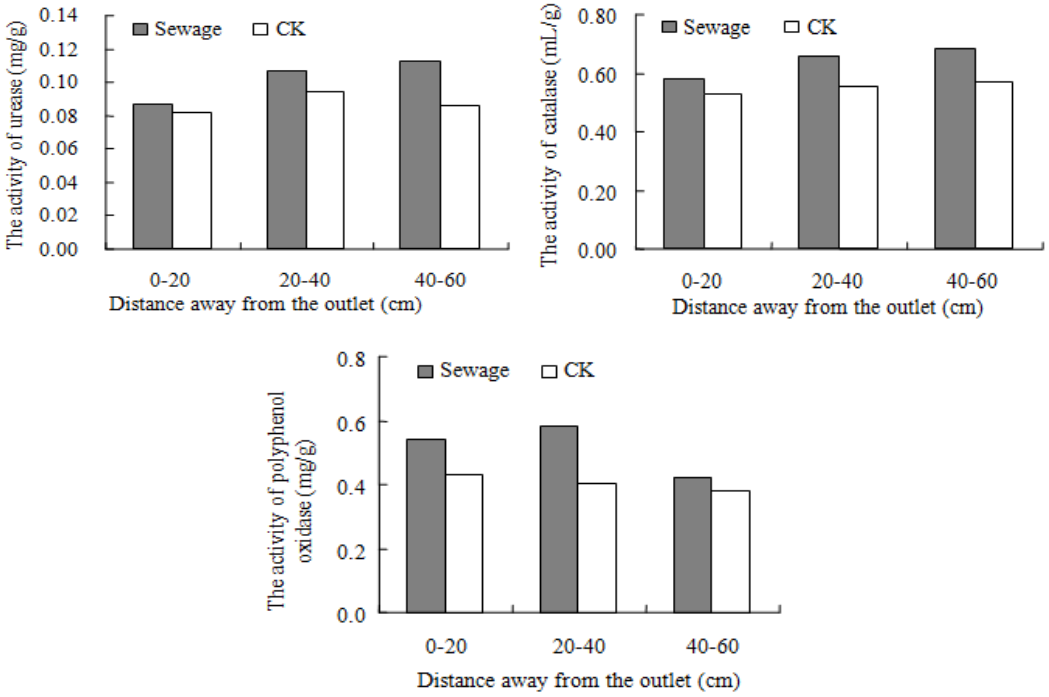

Fig. 3: The enzymatic activities of soils with different distance away from the outlet

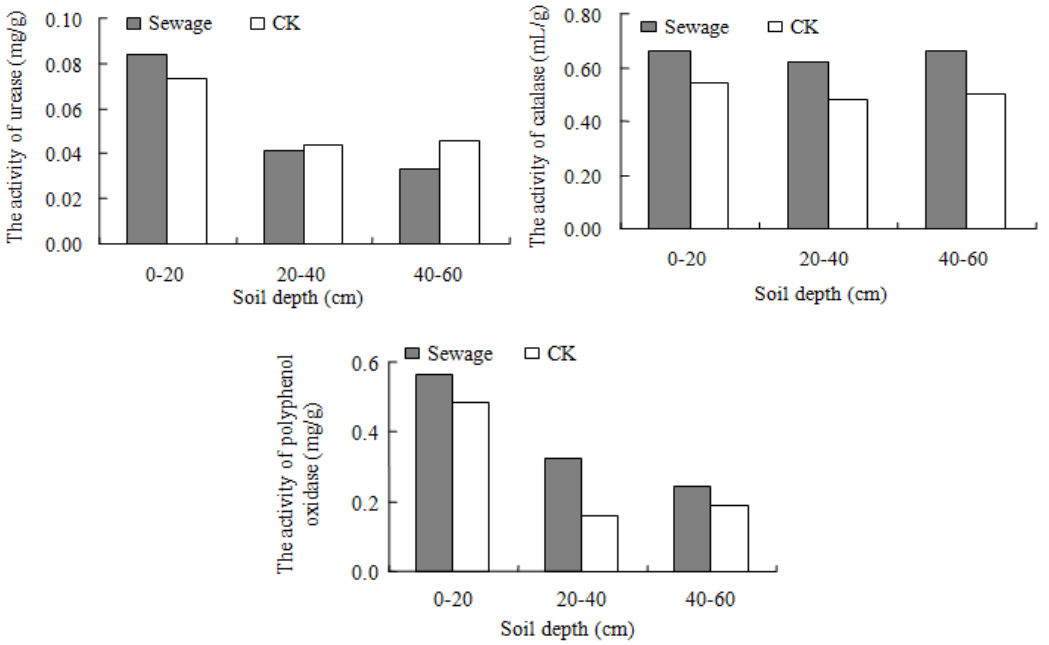

Fig. 4: The enzymatic activities of soils with different depth

Soil enzymatic activities: Figure 3 showed the enzymatic activity of soils with different distance away from the outlet. In general, the sewage increased the enzymatic activities of soils at different distances away from the outlet obviously; the increase extent of urease activity in the soils of 40~60 cm distance was especially significant under the action of sewage, besides, the polyphenol oxidase activity in the soils of 20~40 cm distance was also significantly improved by the sewage.

Figure 4 showed the enzymatic activity of soils with different depth, the urease activity and the polyphenol oxidase activity of soils decreased with the increasing of soil depth, while the variation regularity of soil catalase activity with soil depth was unobvious. On the other hand, the sewage increased the urease

Table 1: Tobacco quality indexes and their values under different treatments (Values in the same column with same letters in the same test item show no significance (Duncan, 5 %))

Treatment	Nicotine (mg/g)	Total N (mg/g)	Reducing sugar (mg/g)	Potassium (mg/g)	Sugar/ nicotine ratio	Nitrogen/ nicotine ratio	Chlorine/potassium ratio
T1	28.21a	25.98a	196.76c	24.18ab	6.97	0.92	11.92
T2	25.44b	25.22a	204.55b	25.22a	8.04	0.99	9.94
T3	25.48b	21.25b	219.87ab	23.19b	8.63	0.83	12.40
T4	22.16c	21.98b	227.23a	23.05b	10.25	0.99	12.62
CK	22.33c	21.04b	220.66ab	21.98c	9.88	0.94	12.95

Table 2: The most appropriate zone of quality index values for flue-cured tobacco leaves

Indexes	Nicotine (mg/g)	Total N (mg/g)	Reducing sugar (mg/g)	Potassium (mg/g)	Sugar/nicotine ratio	Nitrogen/ nicotine ratio	Chlorine/potassium ratio
Range	22~28	20~25	180~220	≥25	8.5~9.5	0.95~1.05	≥8.0

Table 3: Weight coefficient and contribution rate of main ingredients

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	Eigenvalue	r_c/%	r_T /%
f_1	0.259	-0.936	0.984	0.882	-0.426	0.060	-0.804	3.520	50.283	50.283
f_2	0.961	0.287	-0.107	0.225	0.862	-0.224	-0.415	2.033	29.050	79.333
f_3	-0.096	0.112	-0.029	0.310	-0.203	0.962	-0.426	1.267	18.105	97.438

activity in 0~20 cm soil layer but decreased which in 20~40 cm and 40~60 cm layer. The effects of sewage on the activity of soil catalase and polyphenol oxidase showed obvious regularity, the soil catalase and polyphenol oxidase activity of CK was significantly lower than that of sewage treatment, indicating that the sewage had great effects in increasing the soil catalase and polyphenol oxidase activity.

Flue-cured tobacco quality: Table 1 displayed the tobacco quality indexes and their values under different treatments. The nicotine content in flue-cured tobacco leaves had an obvious positive relationship with the total N content, but which had a positive relationship with the reducing sugar content. The sewage treatments had some effects on different indexes of tobacco quality: treatments with higher sewage proportion increased the content of nicotine, total N and potassium in tobacco leaves, but decreased the content of reducing sugar, this regularity was especially found in T1, the content of nicotine and total N in flue-cured tobacco leaves of T1 was significantly higher than that of CK, while the content of reducing sugar in flue-cured tobacco leaves of T1 was significantly lower than that of CK. Table 2 showed the most appropriate zone of quality index values for flue-cured tobaccos, the flue-cured tobacco quality could be assessed by comparing the measured value and the appropriate zone. However, the merits of one quality index could not reflect the comprehensive quality of flue-cured tobacco leaves, a comprehensive and systematic evaluation of flue-cured tobacco quality under different treatments was needed in this study. Hereafter, the Principle Component Analysis (*PCA*) model was introduced to realize the comprehensive evaluation of tobacco quality and the selection of best sewage dilution proportion.

Selection of the best dilution proportion: The principle component of the indexes of flue-cured tobacco quality in Table 1 was extracted following the

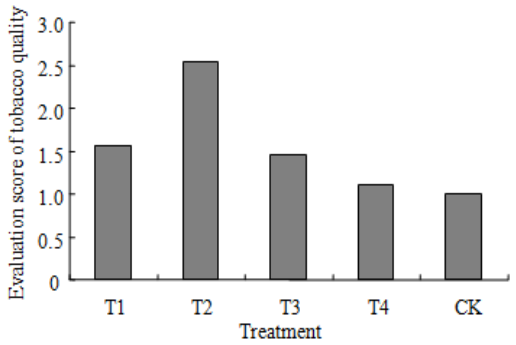

Fig. 5: The quality evaluation score of flue-cured tobaccos under different dilution proportions of sewage based on the calculations of PCA model

principle of "eigenvalue>1, accumulative contribution rate >80%" (Babaoğlu *et al*., 2010; Ul-Saufie *et al*., 2013). However, before the extracting, indexes in Table 1 needed to be converted since the appropriate range shown in Table 2 were "interval index" and which could not be calculated by the PCA model, supposing that the center value of the range in Table 2 was optimal, so the closer the measured value was away from the center value, the better the measured value would be, in the other words, a better measured index would obtain the smaller value of |measured value-center value|. Then the original indexes in Table 1 could be converted to new evaluation indexes: nicotine* = |measured value-25|, total N* = |measured value-22.5|, reducing sugar* = |measured value-200|, sugar/nicotine ratio* = |measured value-9|, nitrogen/nicotine ratio* = |measured value-1|. Using SPSS 17.0 software to calculate the principle component of the sample converted and the calculated eigenvalue, eigenvector, contribution rate (r_c, %) and cumulative contribution rate (r_T, %) were shown in Table 3. Known from the information in Table 3, the first principle component (f_1) reflected the evolution information of total nitrogen (X_2), reducing sugar (X_3), potassium (X_4) and chlorine/potassium ratio (X_7); the

second principle component (f_2) reflected the evolution information of nicotine (X_1) and sugar/nicotine ratio (X_5); the third principle component (f_3) reflected the evolution information of nitrogen/nicotine ratio (X_6) and the cumulative contribution rate reached a high value of 97.438%, which remained vast quantities of the original information. The evaluation score of comprehensive quality of flue-cured tobacco leaves under different sewage treatments was displayed in Fig. 5. Based on the principle of "Better treatment got higher evaluation score", T2 appeared to obtain better overall tobacco quality compared to other treatments on account of higher evaluation score, indicating that T2 was more conductive to flue-cured tobaccos for the equilibrium absorption of nutrients.

CONCLUSION

- The sewage treatment had little effect on surface soil microbial number, but which had some effects on the deeper soil layer, this presented in that the sewage significantly increased the number of bacteria, fungus and actinomyce in 20~40 cm and 40~60 cm soil layer.
- The sewage treatment significantly increased the soil enzymatic activity including the urease activity, catalase activity and polyphenol oxidase activity at all measuring points.
- Treatments with higher sewage proportion increased the content of nicotine, total N and potassium in flue-cured tobacco leaves, but decreased the content of reducing sugar. Based on the calculations of PCA model, the quality sequence from excellent to inferior could be ordered as: T2>T1>T3>T4>CK, to flue-cured tobaccos, T2 was more conductive for equilibrium absorption of nutrients compared with other treatments. On the whole, sewage with 1:2 dilution proportion (T2) was more beneficial for agricultural recycling in this study.

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