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Characterization of microorganisms in the soils with sewage irrigations

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The purpose of this study was to examine the influence of sewage irrigation on the quantity and distribution of soil microorganisms, and to explore the relationship between the content of soil nutrients and the quantity of different microorganism. The results showed that the quantities of the soil actinomycetes, fungi, nitrite oxidizing bacteria (NOB), nitrate bacteria (NB) and denitrifying bacteria (DB) were reduced, while those of soil bacteria, ammonia oxidizing bacteria (AOB) and aerobic cellulose decomposing bacteria (CDB) were increased after sewage irrigation. The distribution of fungi, azotobacter (AB) and cellulose decomposing bacteria (CDB) in the soil rhizosphere of soybean, corn and rice were significantly altered after sewage irrigation. The quantity of soil microorganism in the soil after sewage irrigations was significantly correlated with the soil content of organic substance, ammonia nitrogen and available phosphorous. These results provided theoretical basis for the application and standard establishment of sewage irrigation in the farmland.

Key words: Sewage irrigation, soil microorganisms, soil nutrient, Kuihe River, farmland.

INTRODUCTION

The use of sewage effluents for irrigating agricultural land is a worldwide practice (Feign et al., 1991; Yadav et al., 2002). It is especially common in developing countries, where water treatment cost is high (Rattan et al., 2005; Masto et al., 2009). The rapid development of agricultural and tourist activities, fast industrialization and urbanization, and poor water management practices have made freshwater availability a limiting factor in China. As there is a gradual decline in availability of fresh water for irrigation in China, the use of sewage and other industrial effluents for irrigating agricultural lands is on the rise (Feng et al., 2003; Liu et al., 2005).

With the increasing environmental pollutions, available water resources have been decreasing, which poses a serious threat to the agricultural water supply. Therefore, some studies have been focused on urban sewage irrigations used for farmland (Orchard, 1978). For the

farmers, opportunities exist as sewage effluents from domestic origin are rich in organic matter and also contain appreciable amounts of major and micronutrients. Accordingly nutrient levels of soils are expected to increase with continuous irrigation with sewage water (Yadav et al., 2002). Although there is a strong possibility of agronomic and economic benefits of waste water irrigation; however, in the long-term pollutants could be slowly introduced and accumulated in the soils and cause a potential risk to soil quality and productivity (Friedel et al., 2000).

More and more studies have been conducted on the application of sewage irrigation in agricultures. Some study conducted have shown that the application of sewage water to irrigate sandy soils has a marked effect on improving the soil texture and structure and increasing the water-holding capacity, the carbon content and soil aggregation, while decreasing the bulk density and improving the hydraulic conductivity (Horswell et al., 2003; Liu and Xu, 2002). Sewage irrigation can alleviate the shortage of water, protect water resources, and increase the crop yields. However, long-term sewage

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irrigation may result in the damage of the crops the decline of the soil environment and underwater quality. Different types of crops and irrigations require different quality of water (Mancino and Pepper, 1992; Scott et al., 2003). In China, studies in these areas are still in the initial stages and the majority of the studies focused on the effect of sewage irrigation on the growth of crops (Feng et al., 2003).

Soil microorganism is one of the most active factors in soils. The quantity and composition of microorganisms, especially those surrounding the crop roots, are closely related to the growth of crops. These microorganisms can directly transform the organic substances surrounding the roots into inorganic materials that can be absorbed by the crops. In addition, they can produce growth-stimulating hormone and antibiotics, thus inhibiting the growth of pathogenic microorganisms and stimulating the crop growth (Microbial room Nanjing soil institute of Chinese Acad Sci, 1985).

This study was aimed at:

1. Examining the effect of sewage irrigation on the quantity and distribution of soil common microorganisms including bacteria, actinomycetes and fungi and functional microorganisms including nitrite oxidizing bacteria (NOB), nitrate bacteria (NB), ammonia oxidizing bacteria (AOB), denitrifying bacteria (DB), azotobacter (AB) and cellulose decomposing bacteria (CDB).
2. Exploring the relationship between the content of soil nutrient and the quantity of microorganisms in the soils with sewage irrigations.
3. Attempting to provide theoretical basis for the application of sewage irrigation and the establishment of its standard.

MATERIALS AND METHODS

Description of the studying areas

The areas selected for study were irrigated by the water from Kui River, China, which originates from Hanwang village, Tongshan County, Xuzhou City and runs through the downtown of Xuzhou City via Yunlong Lake Reservoir. The water resource of Kui River is mainly from the industrial wastewater of 130 factories and sewage of 340 000 urban residents because the upstream reservoir is usually closed for impoundment. Daily wastewater volume is approximately 8 to 10 tons. In the dry season, the downstream of the Kui River is closed for impoundment and the untreated sewage is directly used for agricultural irrigation. The lands that use Kui River sewage for irrigation include Shanbao Village of Tongshan County of Xuzhou City, Su County of Anhui Province, etc. The history of sewage irrigation has more than 20 years of history.

Sample collection

The experimental site (sewage irrigation area) was located in Shanbao Village of Xuzhou City and the control site (fresh water irrigation area) was located in the Dalongkou area. Both areas had fluvo-aquic soils. Three types of soils (corn, soybean and rice rhizosphere soils) were included in the experimental land. Soil

microorganisms and nutrients were studied in three sampling areas of each type of soils. In the areas close to the plant roots, the surface soils (upper 0 to 5 cm) were removed and the soils under the surface of 5 to 20 cm were collected at each site. The soil samples (1 kg) were mixed and placed in sterile plastic bags and transported to the laboratory for preservation in refrigerator (4°C).

Dilution of soil samples

An aliquot of 10 g (fresh weight) of the mixed material from each soil was suspended in 90 ml sterile 0.9% sodium-chloride solution. The soil suspensions were shaken for 30min on a gyratory shaker (150 rpm) at room temperature (20°C) for desorption of bacterial cells. The soil suspension was allowed to settle for 1 min, before the supernatant containing the desorbed bacterial cells was decanted into 250 ml sterile Erlenmeyer flasks. From the supernatant a series of 10^{-1} dilutions (highest dilution step: 10^{-10}) was prepared. Those were performed in sterile conditions to avoid the contamination.

Determination of the quantity of common soil microorganisms

The quantity of common soil microorganisms including bacteria, actinomycetes and fungi was determined by plate counting method. The series of soil diluents with 1 ml was taken for inoculating into the tube liquid medium, dilution of soil for bacteria and actinomycetes was from 10^{-5} to 10^{-7} and fungi was from 10^{-3} to 10^{-5}. Three repeats were performed in each dilution, and blank was set to test whether the medium had been contaminated. Bacteria were cultured in beef extract peptone medium at 37°C for 3 days, actinomycetes were cultured in modified Actinomycetes culture medium at 37°C for 3 days, and fungi were cultured in wort medium (100 ml culture with one drop of lactic acid) at 25°C for 5 days. After ending up the incubation, we removed the flat dish, chose proper dilution for counting the colony, and obtained the average colony count. The value was calculated by the following formula:

$$C = ND / W$$

Where, C (cfu g^{-1}) is the colony count per gram dry soil, N (cfu) is the average colony count in the same dilution, D is the dilution multiple with all colony appeared, and W (g) is the quality of dry soil.

Determination of quantity of soil functional microorganisms

The quantity of functional microorganisms including nitrite oxidizing bacteria (NOB), nitrate bacteria (NB), ammonia oxidizing bacteria (AOB), denitrifying bacteria (DB), azotobacter (AB) and cellulose decomposing bacteria (CDB) was determined by most probable number (MPN) method (Yang, 2002; Microbial room Nanjing soil institute of Chinese Acad Sci, 1985). The series of soil diluents with 1 ml was taken for inoculating into the tube liquid medium, five repeats were performed in each dilution, and blank was set to test whether the medium had been contaminated.

Dilution of soil for NOB and NB was from 10^{-2} to 10^{-7}, and was cultured by Stephenson media A and B respectively at 28°C for 14 days. Dilution of soil for AOB was from 10^{-6} to 10^{-9}, and was cultured by ammoniated peptone medium at 28°C for 7 days. Dilution of soil for DB was from 10^{-4} to 10^{-8}, and was cultured by Giltay medium at 30°C for 14 days. Dilution of soil for AB was from 10^{-2} to 10^{-6}, and was cultured by modified Ashby medium without nitrogen at 28°C for 7 or 14 days. Dilution of soil for CDB was from 10^{-1} to 10^{-5}, and was cultured by Hirsch Johnson's medium at 28°C for 14 days (Li et al., 1996). After incubating, we took the number of tubes in which characteristic reaction appeared in the last three concentrated

Table 1. Quantity of common microorganisms in sewage irrigation and fresh water irrigation (control) soils, CFU g^{-1}(dry weight).

Irrigation type		Bacteria	Actinomycetes	Fungi
Fresh water irrigation	1	1.62×10^7	2.58×10^5	1.67×10^3
	2	9.21×10^6	8.67×10^6	6.51×10^4
	3	1.31×10^6	2.09×10^6	8.00×10^4
	Average (K_0)	8.91×10^6	3.67×10^6	4.89×10^4
Sewage irrigation	1	2.26×10^7	2.83×10^5	3.68×10^3
	2	1.62×10^7	5.00×10^6	1.76×10^4
	3	1.25×10^6	1.36×10^6	8.70×10^3
	Average (K_1)	1.34×10^7	2.21×10^6	9.99×10^3
K_1-K_0		4.49×10^6	-1.46×10^6	-3.89×10^4

dilution as microbial growth index. The approximation of most probable number could be found out in the reference (Microbial room Nanjing soil institute of Chinese Acad Sci, 1985), and the value was calculated by the following formula:

$$C = MD / W$$

Where, C (cfu g^{-1}) is the colony count per gram dry soil, M is the approximation of most probable number, D is the highest dilution multiple with all colony appeared and W (g) is the quality of dry.

Analysis of soil nutrients

Soil organic substance was determined by potassium dichromate-sulfuric acid oxidation method. Total nitrogen (N) was determined by semi-micro Kjeldahl method. Total phosphorus (P) was determined by sulfuric acid-perchloric acid-molybdenum anti-colorimetry. Ammonia nitrogen was measured by phenol-hypochlorite colorimetry. Available phosphorus was determined by sodium bicarbonate assay (Chen, 1989; Editorial committee of China's environmental protection agency, 2002).

Statistical analysis

T test was performed to determine the effects of sewage treatment on microorganism quantity. For statistical analysis of data (principal component analysis (PCA) correlations), Microsoft Excel and SPSS window version 13.0 (SPSS Inc., USA) packages were used.

RESULTS AND DISCUSSION

Variation of common microorganism in soils with sewage irrigations

In sewage irrigated soils, the quantity of bacteria was increased by 50.39% (Table 1) and was significantly different in comparison with control, shown by statistical analysis ($P<0.05$), while quantity of actinomycetes and fungi were decreased by 39.78 and 79.55% respectively, having no significant difference. Compared to tap-water irrigation, amounts of bacteria, actinomyces and fungi in

irrigated soils with reclaimed-water were increased especially bacteria (Zhang et al., 2009). By analyzing the soil microflora in sewage and fresh water irrigated soils respectively, we found that there was no difference on quantities of bacteria, actinomyces and fungi (Yuan et al., 2003). Pollutants concentration of sewage, effects of chemical degradation or microbial decomposition on pollutants and the other chemical or biological components in soils might lead to different research results.

Variation of functional microorganisms in soils with sewage irrigations

In comparison with the control soils, the quantity of NOB, NB and DB in sewage irrigated soils were decreased by 63.64, 87.50 and 36.76% respectively having no significant difference shown by statistical analysis (Table 2). The quantity of AOB, AB and CDB was significantly different than control soils, having 100, 233.33 and 364.71% increased respectively as shown by statistical analysis of ($P<0.01$) for AOB and ($P<0.05$) for CDB.

Along with the concentration of beer wastewater increasing continuously, the quantities of aerobic cellulose decomposing bacteria, nitric acid bacteria, nitrous acid bacteria and free-living nitrogen fixing bacteria, all presented the law of first increasing and then decreasing (Shang et al., 2007). Population of nitrogen-fixing bacteria was decreased with long-term organic sewage irrigation then replaced with fresh water irrigation and the populations were gradually increased. Because of the effects of long-term organic sewage irrigation, it could not make nitrogen-fixing bacteria populations recover fully, even for 30 years or longer (Zhang et al., 2007). Long-term sewage irrigation would lead to great decrease of soil microbial biomass and population, recession of community structure and decline of diversity, and then cause disorder of soil structure and function, disruption of ecological balance and so on.

Table 2. Quantity of functional microorganisms in sewage irrigation and fresh water irrigation (control) soils, CFU g^{-1}(dry weight).

Irrigation type		NOB	NB	AOB	DB	AB	CDB
Fresh water irrigation	1	2.81×10^4	1.62×10^5	5.00×10^6	1.00×10^6	5.01×10^4	5.11×10^4
	2	9.12×10^4	9.50×10^6	5.53×10^6	9.10×10^5	4.81×10^3	1.80×10^2
	3	1.32×10^4	2.11×10^4	5.93×10^6	1.32×10^5	2.70×10^4	1.00×10^3
	Average (K_0)	4.41×10^4	3.21×10^6	5.52×10^6	6.81×10^5	2.71×10^4	1.71×10^4
Sewage irrigation	1	1.90×10^4	8.32×10^4	6.23×10^6	3.41×10^5	1.61×10^5	1.92×10^6
	2	2.51×10^4	1.00×10^6	1.94×10^7	5.93×10^5	9.80×10^4	5.80×10^2
	3	3.52×10^3	2.21×10^4	6.51×10^6	3.52×10^5	1.11×10^4	2.11×10^4
	Average (K_1)	1.61×10^4	3.71×10^5	1.12×10^7	4.32×10^5	9.01×10^4	6.41×10^5
K_1-K_0	K_1-K_0		-2.80×10^6	5.50×10^6	-2.49×10^5	6.30×10^4	6.20×10^5

NOB, Nitrite oxidizing bacteria; NB, nitrate bacteria; AOB: ammonia oxidizing bacteria; DB, denitrifying bacteria; AB, azotobacter; CDB, cellulose decomposing bacteria.

Effect of sewage irrigation on the quantity of common microorganisms in the rhizosphere soil of different crops

The rhizosphere microflora of soybean was affected by sewage irrigation in comparison with control. Statistical analysis showed that quantity of bacteria was higher than the control ($P<0.05$) (Figure 1), while fungi and actinomycetes were almost the same in both. In the rhizosphere of corn, there was decrease in quantity of actinomycetes and fungi (sewage irrigation) than the control while quantity of bacteria was significantly higher in sewage irrigated soil ($P<0.05$) (Figure 1). The microflora of the rhizosphere of sewage irrigated rice was lower than the control. Statistical analysis showed that actinomycetes quantity was significantly different than the control ($P<0.05$) (Figure 1), while bacteria and fungi quantity had no variation. These results suggest that sewage irrigation has significant effect on the quantity of common microorganisms in the rhizosphere soils of different crops. The

quantity of all the common microorganisms in the soybean rhizosphere soils has an increasing trend in the sewage irrigation group, while the quantity of all the common microorganisms in the rice rhizosphere soils has a declining trend in the sewage group. Different common microorganisms in the corn rhizosphere soils vary differently upon sewage irrigation.

Irrigated with oily sewage in paddy soil of Shenfu area of China, the amounts of aerobic heterotrophic bacteria and fungi in rice rhizosphere soil were increased according to pollution levels, while the actinomycetes amounts changed little (Li et al., 2005). In comparison with tap-water irrigation, the numbers of bacteria, actinomyces and fungi were lower in Kentucky bluegrass soil after irrigated by tertiary effluent for years, but higher in Tall Fescue soil (Han et al., 2006). The great differences of microbial quantities and species in plant rhizosphere soils with sewage irrigation were due to plant kinds, soil types, kinds and concentration of pollutants and so on.

Effect of sewage irrigation on the quantity of functional microorganisms in the rhizosphere soil of different crops

In the rhizosphere of sewage irrigated soybean, the quantity of DB was significantly lower ($P<0.05$) (Figure 2) while that of AB and CDB was higher ($P<0.05$) (Figure 2) and that of NOB, NB and AOB remain the same as compared to control. There was significant decrease ($P<0.05$) (Figure 2) in the quantity of NOB and NB in the rhizosphere soils of corn having sewage irrigation while quantity of DB remain almost the same. The quantity of AOB was higher ($P<0.05$) (Figure 2) while that of AB and CDB was extremely higher than the control ($P<0.01$) (Figure 2). The quantity of DB in the rhizosphere soils of rice was significantly increased ($P<0.05$) (Figure 2) after sewage irrigation while that of NOB and AB become lower ($P<0.05$) (Figure 2) as compared to control. After sewage irrigation the quantity of CDB was much higher ($P<0.01$) while NB and AOB remains the same.
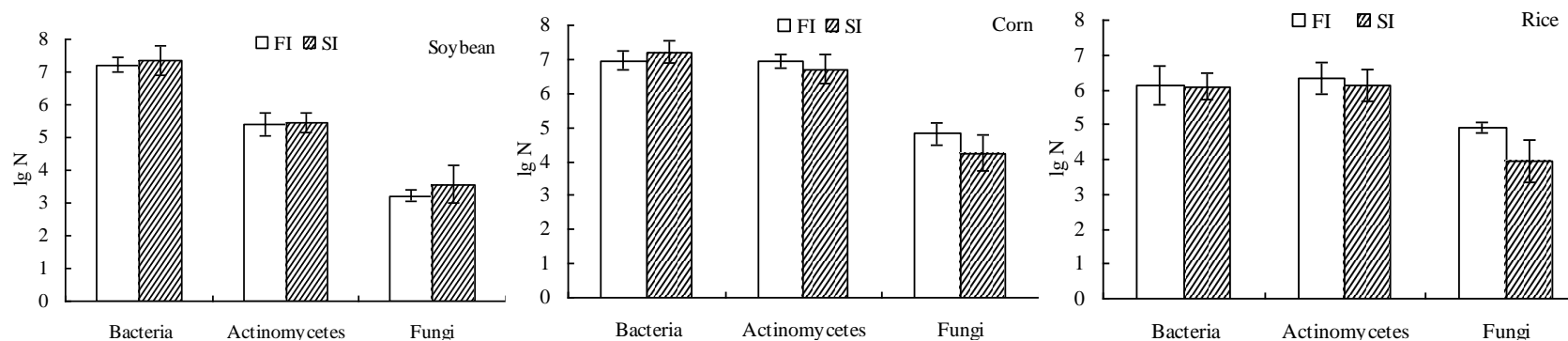
Figure 1. Change of common microorganism quantity in soybean rhizosphere soil (top panel), corn rhizosphere soil (middle panel) and rice rhizosphere soil (bottom panel). FI, Fresh water irrigation; SI, sewage irrigation. X-axis represents different microorganism, Y-axis represents the quantity of the microorganisms.
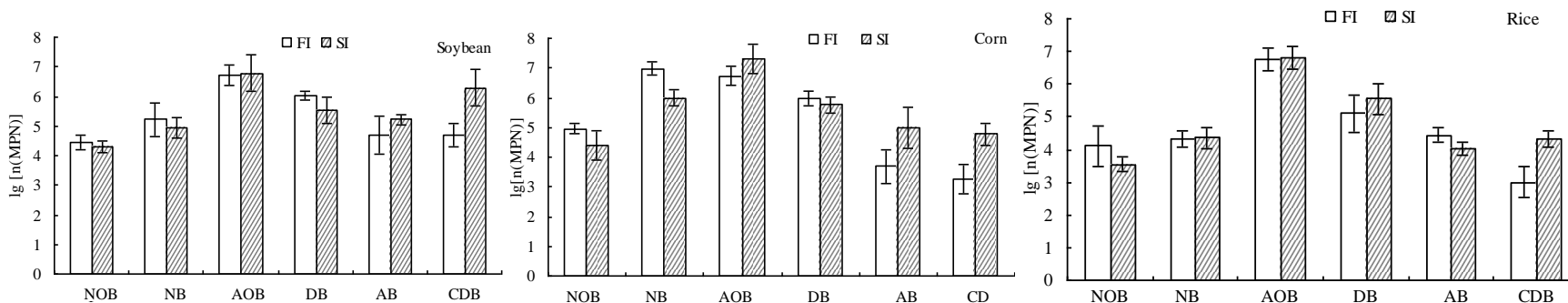

Figure 2. Change of functional microorganism quantity in soybean rhizosphere soil (top panel), corn rhizosphere soil (middle panel) and rice rhizosphere soil (bottom panel). FI, Fresh water irrigation; SI, sewage irrigation; NOB, nitrite oxidizing bacteria; NB, nitrate bacteria; AOB, ammonia oxidizing bacteria; DB, denitrifying bacteria; AB, azotobacteria; CDB, cellulose decomposing bacteria. X-axis represents different microorganism, Y-axis represents the quantity of the microorganisms.

These results suggest that sewage irrigation has large effect on the quantity of functional microorganisms in the rhizosphere soils of different crops. The quantity of AB and CDB is significantly different between the sewage irrigation group and the control group, indicating that sewage irrigation largely affects the quantity of AB and CDB. In addition, CDB quantity has an increasing trend in the sewage irrigation group, indicating that sewage irrigation is favorable for the proliferation of CDB. The influence of long-term sewage irrigation on microbial populations and major microbial physiological groups of wheat rhizosphere was obvious, the numbers of bacteria, actinomycete, cellulose decomposing bacteria, azotobacteria, nitrobacteria and ammonifying bacteria were decreased, but fungi and denitrobacteria increased after the long-term sewage irrigation (Ge et al., 2009). Compared with fresh water irrigation, nitrogen-fixing bacteria and nitrifying bacteria numbers in rhizosphere soils of rape and cotton had no significant change (Yuan et al., 2003).

However, long-term sewage irrigation could

Table 3. Correlation between quantity of common micro-organisms and nutrients in sewage irrigation soils.

Item	Total nitrogen	Ammonia Nitrogen	Total phosphorous	Available phosphorous	Organic substance
Bacteria	0.144	0.251	0.047	-0.075	0.843*
Actinomycetes	-0.366	-0.117	-0.921**	0.005	0.122
Fungi	-0.532	0.112	-0.283	-0.100	0.139

Table 4. Correlation between quantity of functional microorganisms and nutrients in sewage irrigation soils.

Items	Total nitrogen	Ammonia nitrogen	Total phosphorous	Available phosphorous	Organic substance
NOB	0.981	0.973*	-0.234	-0.571	0.928
NB	0.926	0.688	-0.729	-0.928	0.571
AOB	0.718	-0.367	-0.932	-0.967*	0.223
DB	0.715	0.364	-0.933	-0.988*	0.220*
AB	0.883	0.988*	0.059	-0.307	0.996
CDB	0.414	0.752	0.655	0.337	0.843*

NOB, Nitrite oxidizing bacteria; NB, nitrate bacteria; AOB, ammonia oxidizing bacteria; DB, denitrifying bacteria; AB, azotobacteria; CDB, cellulose decomposing bacteria.

cause seriously soil pollution. These pollutants could easily accumulate in soils and be absorbed by the crops. Subsequently, they entered human body through food chain and caused health problems to humans. Vegetables (for example, spinach, beet) irrigated by sewage had higher NH_4^+-N concentration than the WHO's standard (Xie and Ma，2001). Compared to control, people in sewage irrigation area were higher incidence of diarrhea and digestive system cancer and lower repair capability of human DNA (Huang and Wang, 2009).

Relationship between the content of soil nutrients and the quantity of common microorganisms

The quantity of bacteria was positively correlated with soil total nitrogen, ammonia nitrogen and organic substances (Table 3). In addition, the quantity of bacteria was significantly correlated with organic substance (r = 0.843), which is consistent with previous studies on the relationship between the soil nutrition and the quantity of microorganisms (Huang et al., 2007). The quantity of actinomycetes was negatively correlated with the total phosphorus (r = -0.921). Significant correlation was not observed between the quantity of fungi and soil physical and chemical properties. In irrigation with regenerated sewage, there were significant correlations between bacteria, actinomyces amounts and organic matter, effective phosphorus contents, and between actinomyces amounts and conductivity values, but the other correlations were not significant (Jiao et al., 2010). For a

certain relationship between microbial quantities and soil nutrients, the microbial quantities could be used as one of evaluation indicators for fertility changes in sewage irrigation soils.

Relationship between the soil properties and the quantity of functional microorganisms

The quantity of NOB and NB was positively correlated with total nitrogen and ammonia nitrogen (Table 4). In addition, NOB quantity was significantly correlated with ammonia nitrogen. Soil NH_4^+-N provided sufficient substrates for NOB, which facilitated the proliferation of NOB. Thus, the content of NH_4^+-N in the soils was the key factor affecting the quantity of NB. AOB in the soils can mineralize organic nitrogen into NH_4^+-N, which can be absorbed and utilized by plant and microorganisms. The quantity of AOB was negatively correlated with the content of available phosphorus (r =-0.967), which might be due to the consumption of available phosphorus by AOB and DB during their growth and metabolic process. The quantity of DB and CDB was positively correlated with the content of organic substance, because the organic substance-rich, energy sufficient soils can effectively facilitate the proliferation of DB and CDB (Song et al., 2002) which is beneficial to soil fertility.

In Shenyang in China, it found that there was a significant positive correlation between soil nutrients (for example organic carbon, N, P, K) and functional microorganisms' parameter, so soil nutrients of sewage irrigation area had a positive effect on the microorganism growth (Zhang et al., 2006). But the effect of long-term

sewage irrigation on soils was great, and might change the structure and function of soils (Ge et al., 2009).

Conclusion

Concerning the common microorganism, sewage irrigation by the water from Kui River can significantly increase the quantity of bacteria and reduce, though not significantly, the quantity of actinomycetes and fungi. For the functional microorganisms, sewage irrigation can reduce the quantity of NOB, NB and DB, and increase the quantity of AOB, AB and CDB. The changes of AOB and CDB after sewage irrigation were significant ($P<0.05$) and extremely significant ($P<0.01$), respectively. The distributions of fungi, AB and CDB in the rhizosphere soil of soybean, corn and rice were significantly altered after sewage irrigation. The quantity of bacteria was significantly correlated with the content of organic substance ($r = 0.843$). The quantity of actinomycetes was negatively correlated with the content of total phosphorus ($r =- 0.921$). The quantities of NB and AB were positively correlated with the content of ammonia nitrogen ($r = 0.973$ and $r = 0.988$, respectively). The quantity of AOB and DB was negatively correlated with the content of available phosphorus ($r = -0.967$ and $r = -0.988$, respectively). The quantities of DB and CDB were positively correlated with the content of organic substances ($r = 0.220$ and $r = 0.843$, respectively).

Generally, the important relationship between microbial species, quantity and soil's function, structure of in sewage irrigation areas should be recognized. By determining the microbial species and numbers, the soil conditions of function and structure might be predicted and it would provide further theoretical data for applying sewage irrigation in farmlands (Jiao et al., 2010).

ACKNOWLEDGEMENTS

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