Effects of compound microbial inoculant treated wastewater irrigation on soil nutrients and enzyme activities

Wang Chunfang[1], Shao Xiaohou[1*], Xu Huilian[2], Chang Tingting[1], Wang Weina[1]

(1. *Key Laboratory of Irrigation-Drainage and Agricultural Water and Soil Environment in Southern China, Ministry of Education, Hohai University, Nanjing 210098, China*; 2. *International Nature Farming Research Center, Matsumoto, Nagano 390-1401, Japan*)

Abstract: Wastewater treated by compound microbial inoculant (CMI) in agricultural irrigation can enrich soil fertility and decompose the possible pollutants. In this study, a greenhouse experiment using tomato as the model crop was performed to investigate the effects of treated wastewater irrigation on soil nutrients and enzymes. For this purpose, certain parameters were measured, including soil total nitrogen (N), nitrate N, total phosphorus (P), available potassium (K)and the activities of the enzymes urease, acid phosphatase and catalase in soils irrigated with fresh water, wastewater and CMI-treated wastewater under three amount of irrigation water. The results showed that irrigation with both treated and untreated wastewater significantly increased soil total N, total P, and available K, however the treated wastewater showed higher effects on soil enrichment, especially on available K. The activity of soil urease and acid phosphatase reached highest with treated wastewater irrigation, whereas wastewater irrigation increased the activity of catalase obviously. Soil enzyme and nutrient with fresh water irrigation decreased with increasing water amount; the content of soil urease, nitrate-nitrogen, total N and total P in treated wastewater and wastewater irrigation rose with increasing water amount, but the highest activity of acid phosphatase and the lowest activity of catalase were found in medium irrigation water amount. Under the condition of tomato cultivation, total N, nitrate N and total P were closely correlated with soil urease and catalase; there were significant positive correlation among soil urease, catalase, total N, nitrate N and total P; there existed significantly positive correlation between acid phosphatase and all measured soil nutrient indexes. The results suggested that irrigation with CMI-treated wastewater is a security and effective strategy to agricultural land management.

Keywords: wastewater irrigation, water quality, soil enzyme activity, soil nutrients, compound microbial inoculant (CMI)
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1 Introduction

Wastewater irrigation can alleviate the contradiction between supply and demand of water resources[1,2]. With the rapid development of intensification of livestock and poultry husbandries, the treatment and recycling of wastewater are gaining widespread attention worldwide, especially in developing countries. The husbandry wastewater is rich in N, P, K and other nutrients which are indispensable for crop growth, and thus it can be reused for agriculture irrigation. However, the untreated wastewater contains substantial amount of organic matter, salts, suspended solids, pathogens and bad smell, which are harmful to soil and environment[3-5]. A number of methods were in practice for purification of water and removing the pollutant contaminants. One of the promising ways in improving water quality is microbial technology, which has been much more appreciated comparative to other conventional methods because of its

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Biographies: Wang Chunfang, PhD candidate, research interests: agricultural water and soil engineering, Email: sk0602wcf@163.com; **Xu Huilian**, Professor, research interests: water-saving irrigation and efficient use of water resources, Email: huilian@infrc.or.jp; **Chang Tingting**, PhD candidate, research interests: agricultural land and water engineering, agricultural irrigation technology, Email: changtingting225@163.com; **Wang Weina**, Master, research interests: agricultural water and soil engineering, Email: wangweinaqingzhu1110@126.com.

***Corresponding author: Shao Xiaohou,** Professor, research interests: agricultural water and soil engineering. College of Water Conservancy and Hydropower Engineering, Hohai University, Nanjing 210098, China. Tel: +86-13951005013, Email: shaoxiaohou@163.com.

eco-friendly properties and low inputs and cost. In recent years, a compound microbial inoculant called EM (effective microorganisms) has been widely used in crop production and animal husbandry. Wastewater treated by EM and then reused for agriculture irrigation offers the greatest scope for application because it has the potential to meet growing water demands, reduce the use of chemical fertilizers and minimize hazards from toxic contaminants of the soils and agricultural products[6,7].

Soil nutrients and soil enzymes, as the important indexes of soil quality and fertility, drive the process of soil biological metabolism and the comprehensive performances of soil biological activity. Soil nutrients and enzyme activity are important indicators in evaluating the effects of irrigation with reclaimed waters[8,9]. The effects of treated wastewater irrigation on soil physical, chemical and biological properties have been studied by many investigators. Considering that the amount of water used globally for agricultural purposes is increasing while the resources are limited or even diminishing, over the past three decades fresh water is increasingly replaced by treated wastewater to the agricultural irrigation[10]. It has been well established that irrigation with raw wastewater as a risk factor for crop irrigation could decrease soil quality[11]. Apart from the benefit of replacing scarce fresh water resources, irrigation with treated wastewater also provides nutrients and organic matter thus enhancing the nutrient availability and soil fertility[12]. The main nutrients having concentrations in sewage water higher than the original fresh water are usually N, P and K. The wastewater contains N and P in organic forms that do not exist in fresh water. Therefore the plant availability of N and P applied by irrigation with wastewater and their fate in environment are different from those of N and P fertilizers[13,14]. There was an enhancement in the soil enzyme activities following 10 and 20 years of treated municipal wastewater irrigation[15]. Soil enzymes activities are very sensitive to changes in soil status affected by agricultural management practice or pollution, so they have been widely regarded as the indicators of soil environmental quality[16]. Both soil enzyme activity and microbial biomass are important factors of soil quality as involved in nutrient cycling. Since microbial activities are strongly dependent on nutrition content and thus rapidly respond to changes in soil properties[17]. There was a good effect of EM techniques on organic wastewater for agricultural irrigation[18]. To date, the most research on wastewater has been focused on issues related to improvement in crop yields and heavy metal build-up ignoring the dynamics and changes of nutrients and enzymes activity in soils issues[19-22]. In this study, a two factorial experiment with three levels of water quantity and three water quality was conducted to analyze effects on soil nutrients and enzymes activity at different soil depths under conditions of greenhouse tomato cultivation. The primary objective of this research was to evaluate the impact of applicability of CMI-treated wastewater on soil properties, and determine a safe and reasonable irrigation model of reclaimed water.

2 Materials and methods

2.1 Experimental site

The field experiments were conducted at Hengxi farm of Institute of Vegetables and Flowers in Nanjing City (31°57′N, 118°50′E) from May to October in 2013. The local climate is subtropical monsoon climate with ample heat energy and rainfall. The mean annual temperature was 15.7°C and the mean annual precipitation was 1106.5 mm. There were about 237 days annually without frost and the maximum mean humidity is 81%, and the maximum wind velocity is 19.8 m/s. The Meiyu season (East Asian rainy season) happened in June and July. Soil texture of the site was clay yellowish brown loam. The main physical and chemical characteristics of the soil layer of the experimental site (0-20 cm) are as follows: 14.2 g/kg of organic matter, 0.9 g/kg of total N, 0.53 g/kg of total P, 5.87 of pH, 1.35 g/cm^3 of bulk density, 3.6 mg/kg of NO_3^-N, 129.9 mg/kg of alkali-hydrolyzable N, 27.2 mg/kg of available phosphorus, 217.3 mg/kg of available K, 0.163 mg NH_3-N/(g·d) of urease, 0.366 mg Phenol/(g·2h) of acid phosphatase and 3.15 mL 0.1 mol/L $KMnO_4$/(g·h) of catalase.

2.2 Experimental design

Tomato (*Solanum lycopersicum* L. cv. Grand-Red) was used as the model vegetable crop, which were transplanted into the plots in mid-April and harvested in early August. Tomato was grown in a natural lighting polyethylene greenhouse, and there was no rainfall could be considered in the whole stages.

The experiment was designed as three kinds of water quality (fresh water, dairy wastewater and CMI-treated dairy wastewater) with three levels of irrigation amounts (280 mm, 320 mm and 360 mm during the whole growth stage), as shown in Table 1. The fresh water was from a groundwater source that is commonly applied for crop irrigation in the experimental area. The wastewater was taken from the cattle farm of Nanjing Vegetable Science Research Institute, which was diluted 30 times and then irrigated to the soil. For the CMI-treated wastewater, the inoculant was mixed with the 30-time diluted wastewater in the ratio of 3:100 and incubated in airtight plastic tanks for one week. The quality of irrigation water was shown in Table 2. The CMI was supplied by Nanjing EMRO Co. Ltd. The CMI mainly contains yeasts, lactic acid bacteria and photosynthetic bacteria at a total density of 10^{10}/mL with a pH of 3.5 caused by many kinds of organic acids.

The experiment was laid out in a randomized-plot design with all treatments were replicated three times, each plot size was 2 m×4 m. The design of irrigation schedule at different growth stages of tomato is listed in Table 3. The surface water irrigation was adopted, and the adjoining plots were separated by 1 m deep geomembrane to avoid water exchange in horizontal direction. Pest and weed control were performed according to local management practices.

Table 1 Design of different treatments

Treatment	T1-1	T1-2	T1-3	T2-1	T2-2	T2-3	T3-1	T3-2	T3-3
Water	Fresh	Fresh	Fresh	Treated	Treated	Treated	Waste	Waste	Waste
Amount/mm	280	320	360	280	320	360	280	320	360

Table 2 Indices of different irrigation water quality

Water	NO_3^--N/mg·kg^{-1}	NH_4^+-N/mg·kg^{-1}	EC/μs·cm^{-1}	pH	TN/mg·kg^{-1}	TP/mg·kg^{-1}	CODcr
Fresh	8.71	2.12	145	7.89	1.7	15.3	18
Treated	15.57	20.01	149	7.23	6.2	35.3	642
Waste	30.23	1.45	662	7.94	8.6	42.3	3820

Table 3 Design of irrigation schedule at different growth stages

Total amount /mm	Early vegetative growth/mm	Late vegetative growth/mm	Flowering /mm×time	Fruit set /mm×time	Fruit Development /mm×time	Fruit maturity (green)/mm×time	Ripening /mm×time	Irrigation times
280	30	40	10 × 2	10 × 4	10 × 4	10 × 4	35 × 2	18
320	30	40	15 × 2	15 × 4	15 × 3	15 × 3	35 × 2	16
360	30	40	20 × 2	20 × 3	20 × 3	20 × 3	35 × 2	15

2.3 Measurements

After the tomato harvest, soil samples were collected from three sites in each treatment plot at 0-20 cm, 20-40 cm, and 40-60 cm soil layers. Each soil sample was divided into two parts. One was air-dried in room, ground and then passed through 2 mm sieves successively for physical-chemical analyses, and the other was kept fresh at 4°C in a refrigerator for soil enzymatic activities analyses in a week[23,24]. Total nitrogen (TN) was determined by Semimicro-Kjeldahl Method with concentrated sulfuric acid digestion; nitrate (NO_3^--N) was measured using the phenol disulfonic acid colorimetry; total phosphorus (TP) was determined by $HClO_4$-H_2SO_4 method; available potassium (AK) was determined using the flame photometry with ammonium acetate extraction; soil urease (URE) activity was measured using the phenol-sodium hypochlorite colorimetry; acid phosphatase (ACP) activity was determined by disodium phenyl phosphate colorimetry method; and catalase (CAT) activity was determined using the potassium permanganate titration volumetric method.

2.4 Statistical analysis

Data were subjected to One-way analysis of variance (ANOVA) treatments and the method used to discriminate among the means was Fisher′s least significant difference (LSD-test) for $p<0.05$. All Graphics were carried out using OriginPro 8.5, and the Correlate analysis was performed with SPSS 17.0.

3 Results and discussion

3.1 Effect of irrigation water quality and quantity on soil nutrients

3.1.1 Soil total N

As shown in Figure 1, irrigation with both wastewater and treated wastewater significantly increased total N in the soil by 59.1% and 40.5% in the three soil layers compared to control, respectively. The increase in soil N was more in untreated than in CMI-treated wastewater in all the three soil layers, indicating that the wastewater for direct irrigation could cause high levels of nitrogen accumulation, and deterioration of soil quality parameters, however, after treated by CMI, a part of N in wastewater was decreased in order to meet microbial metabolic demands[25].

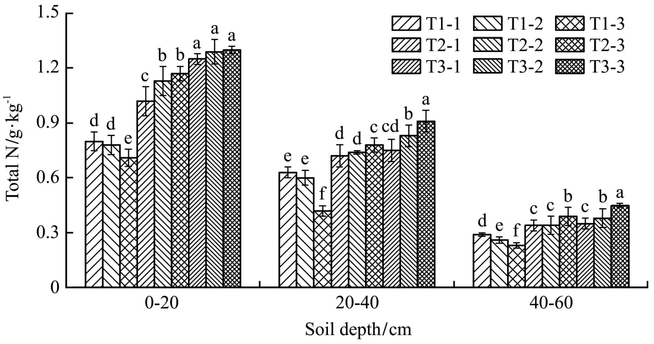

Figure 1 Effect of irrigation water quality and quantity on soil total nitrogen

With increases in irrigation amount, the total N in the soil of fresh water irrigation decreased slightly, which might be due to the leach of some hydrolyzable nitrogen in soil[26,27]. Along with the increase of irrigation water amount, the soil total N increased in the plots of both wastewater and CMI-treated wastewater treatments, which was attributed to the contents of N in the wastewater applied. When the water amount for irrigation was not enough, some fertilizers required for crop growth, while in contrast, excessive water amount for irrigation could lead to environmental pollution[28]. The total N in the same treatment showed a decreasing trend for the deeper soil layers, with significant differences among soil depths.

3.1.2 Soil nitrate N

The nitrate nitrogen was the main form of nitrogen utilization by upland plants, but the excessive accumulation of nitrate N in soil has become one of the potential environmental problems[29]. Figure 2 shows that both untreated and treated wastewater treatments significantly increased soil nitrate N compared to control, with totals of 28.2% and 51.0% in the three soil layers, respectively. Unlike the total N, the increase in nitrate N in CMI-treated was more than that in untreated wastewater irrigation plots. This suggested that the microbial in treated wastewater involved in N mineralization and transformed the organic nitrogen into nitrate[30]. The nitrate N in control plot decreased slightly with the increase of irrigation water amount. Along with the increase in soil depth, the nitrate N for different treatments reduced. The nitrate N was more found in 20-60 cm soil layer, which indicates that the soil nitrate nitrogen was leached from the surface to this layer[31].

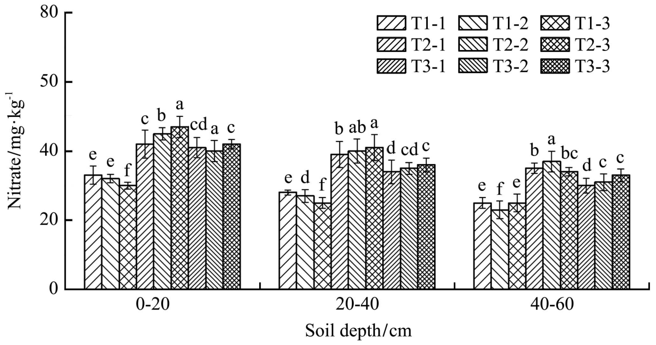

Figure 2 Effect of irrigation water quality and quantity on soil nitrate

3.1.3 Soil total phosphorus

As shown in Figure 3, the total P in untreated and CMI-treated wastewater irrigation plots increased by 47.1% and 29.9% in the three soil layers compared to control, respectively. The total P in CMI-treated wastewater plot was slightly lower than that in untreated wastewater plot, suggesting that the phosphatases in CMI-treated wastewater mainly participate in the hydrolysis of organic phosphorous compounds and transform them into inorganic phosphorous, which were easily up-taken by plants[32].

The soil total P in control plot decreased with the increase of irrigation water amount; meanwhile it increased as irrigation amount increased in both CMI-treated and untreated wastewater plots. With increases in soil depth, the total P showed a decreasing trend.

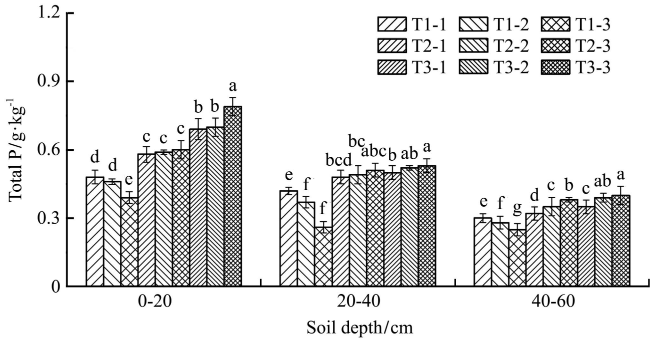

Figure 3 Effect of irrigation water quality and quantity on soil total phosphorus

3.1.4 Soil available K

The soil available K showed direct beneficial effects on plant growth and reflected the supply level of soil K. Figure 4 showed that the soil available K in untreated and treated wastewater irrigation plots increased by 20.3% and 51.1% in the three soil layers compared to control, respectively. The available K in CMI-treated wastewater irrigation plots was significantly more than that in untreated wastewater irrigation plots, suggesting that the soil available K was promoted releasing from the soil by the enzymes in CMI-treated wastewater.

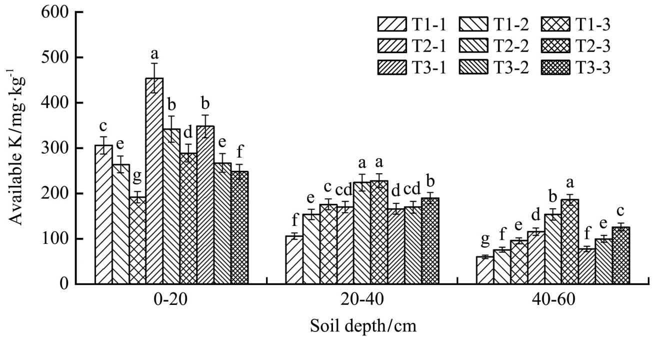

Figure 4 Effect of irrigation water quality and quantity on soil available K

At the 0-20 cm soil layer, with increases in irrigation amount, the soil available K decreased. At the 20-60 cm soil layer, the soil available K increased with increased irrigation amount, which might be due to leaching of the available K[33]. The available K in 0-20 cm soil layer was significantly more than that in 40-60 cm soil layer, indicating that the accumulation of available K was more in the surface soil layer.

3.2 Effect of irrigation water quality and quantity on soil enzyme activities

3.2.1 Soil urease

The urease hydrolyzes urea and exists in most of the bacteria, fungi and higher plants Urease activity commonly characterizes the nitrogen status of soil[34]. Figure 5 shows that irrigation with both wastewater and treated wastewater significantly increased urease activity in the soil by 44.9% and 92.3% in the three soil layers compared to control, respectively. The increase in urease activity in CMI-treated was more than that in untreated wastewater irrigation plots in all the three soil layers, which was attributed to significant increase in microbial biomass inputs by wastewater irrigation.

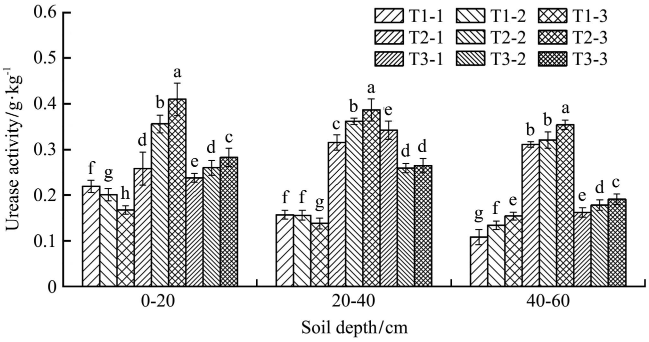

Figure 5 Effects of irrigation water quality and amount on soil urease activity

With increases in irrigation amount, the soil urease activity in the irrigation plots with fresh water showed a decreasing trend. At the same conditions, the soil urease activity increased in plots of both wastewater and treated wastewater treatments. This suggested that the increasing of soil organic matter and nutrient inputs stimulate the activity of enzyme[35]. ANOVA results showed that there was no significantly difference in urease activity at each soil depth. The soil urease was found more in 20-60 cm soil layer, indicating that CMI-treated wastewater and high water amount application had a significant impact on soil urease. In addition, on the condition of shortage of irrigation water, the quality of water may have significant impact on the difference of urease activity; while for enough water amount, the impact of quality of water may be weak, which based on the hypothesis that the microbial population density is limited.

3.2.2 Soil acid phosphatase

The soil phosphatase is divided into acidic, neutral and alkaline. Fungi release more acid phosphatase and bacteria release more neutral phosphatase than others. Phosphatase decomposes varieties of organic phosphorus compounds, providing effective phosphorus for plant growth. Soil phosphatase activity characterizes the status of soil fertility (especially phosphorus status)[36]. As shown in Figure 6, the acid phosphatase activity in untreated and treated wastewater irrigation plots increased by 10.7% and 21.4% in the three soil layers, respectively. The acid phosphatase activity in CMI-treated wastewater plot was slightly more than that in untreated wastewater plot, indicating that the microbial inoculant added in wastewater improved the acid phosphatase activity and enhanced the effectiveness of soil phosphorus. With different irrigation water amount, the highest soil acid phosphatase activity was found in medium irrigation water amount, suggesting that the concentration of microbial communities is limited[37]. Acid phosphatase activity in the same treatment showed a decreasing trend for deeper soil layer, and it was much more in 0-20 cm soil layer than in 20-60 cm soil layer.

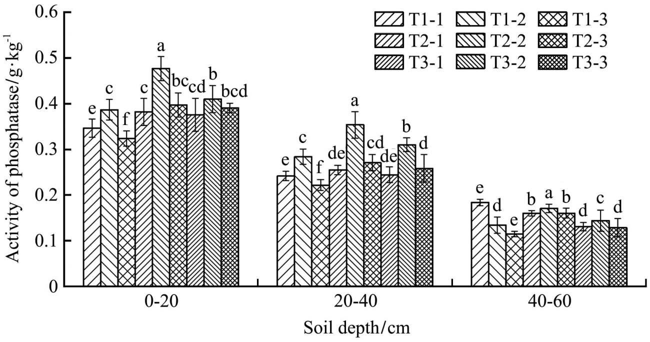

Figure 6 Effects of irrigation water quality and amount on soil acid phosphatase

3.2.3 Soil catalase

The catalase is mainly from exudates of bacteria, fungi and plant root. The accumulation of hydrogen peroxide imposes toxic effects on organisms in the soil. Catalase exists in living organisms and soil can decompose hydrogen peroxide into water and oxygen, removing toxic effects[38]. Figure 7 showed that both untreated and treated wastewater treatments significantly increased soil catalase compared to control by 39.2% and 18.6% in the three soil layers, respectively. The catalase activity was significantly more in untreated than that in CMI-treated wastewater irrigation plots, indicating that wastewater irrigation can lead to toxicity problems by high levels of nutrient accumulation and stimulate the activity of catalase[32].

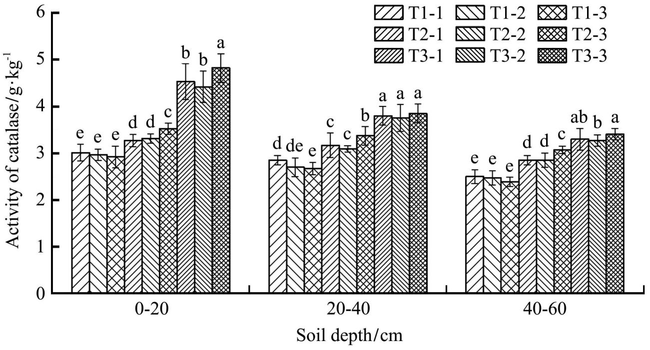

Figure 7 Effects of irrigation water quality and amount on soil catalase

With increases in irrigation amount, the soil catalase in wastewater increased obviously, especially in 0-20 cm soil layers, suggesting that wastewater had significant effects on soil catalase and might exert certain risks on soils and crops if it was directly used for farmland irrigation. The soil catalase activity was relatively lower in medium water amount. There was no significant difference among soil depth levels and with increases in soil depth, the catalase activity showed no substantial changes.

3.3 The correlation between soil enzyme activity and soil nutrients

The analysis of correlation between soil nutrients and enzyme activities is shown in Table 4. The urease had a strongly positive correlation with soil total N, nitrate N and total P (p<0.01). There existed a closely positive correlation between catalase and soil total N, nitrate N and total P (p<0.01). The soil acid phosphatase was correlated with all indexes of the soil nutrient measured (p<0.01). The correlation indicated a significantly positive correlation between soil enzyme activity and soil nutrient. This suggested that soil enzyme activity is an important factor of soil quality as involved in nutrient cycling, and the nutrient inputs stimulate enzyme activities and affect enzyme activity patterns[32,35]. The result was supported by Tao et al.[39].

Table 4 Correlation coefficients between soil enzyme activities and soil nutrients

Index	Urease	Phosphatase	Catalase	Total N	NO_3^--N	Total P	Available K
URE	1.00	0.441*	0.362	0.461*	0.861**	0.488**	0.478*
ACP		1.00	0.528**	0.910**	0.691**	0.818**	0.852**
CAT			1.00	0.792**	0.653**	0.891**	0.462*

Note: * Significance at $p \leq 0.05$; ** Significance at $p \leq 0.01$.

4 Discussion

Irrigation with wastewater and CMI-treated wastewater significantly increased soil nutrients and enzyme activities compared to the fresh water irrigated control. These increases could be attributed to the high organic load of wastewater, which provided large amounts of nutrients to the soils and thus stimulated microbial activity[30]. The soil Total N, NO_3^--N, Total P, Available K and urease activity were enhanced by increasing irrigation water amount, however, acid phosphatase activity was high when medium amount (320 mm) of wastewater was irrigated and the catalase activity was the lowest in the treatment with medium irrigation amount. There were significantly positive correlations between nutrients and enzyme activities in the soils, suggesting that different enzymes in soils play important roles in the transformation of different plant nutrients[40].

It should be mentioned that mismanagement of wastewater irrigation can lead to the toxicity problems of high levels of nutrient accumulation, which deteriorate the soil quality and contaminate environment, and should be avoided for irrigation directly[28].

5 Conclusions

The CMI-treated wastewater irrigation provided the communities of compound microbial inoculants in soil, which not only underpin essential ecosystem functions, including decomposition, mineralization of available nutrients for plant growth, but also reducing the potential of environmental pollution, which could be a relatively irrigation model for ecological intensification of agriculture. In addition, the medium irrigation amount (320 mm) was the most appropriate both for providing nutrients required of the crop and for saving water. The security of reclaimed water application in agricultural production remained to be further studied.

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[References]

[1] Becerra-Castro C, Vaz-Moreira I, Manaia C M, Lopes A R, Silva E F, Nunes O C. Wastewater reuse in irrigation: a microbiological perspective on implications in soil fertility and human and environmental health. Environment International, 2015; 75(4): 117–135.

[2] Qadir M, Wichelns D, Raschid-Sally L, Mccornick P G, Chsel P, Bahri A, et al. The challenges of wastewater irrigation in developing countries. Agricultural Water Management, 2010; 97(4): 561–568.

[3] Zeng X, Liu S, Li Y, Zhang K. Current status and future trend of researches on the irrigation using reclaimed wastewater from livestock and poultry breeding. Journal of Irrigation and Drainage, 2008; 26(6): 1–5.

[4] Castro E, Mañas P, De Las Heras J. Effects of wastewater irrigation in soil properties and horticultural crop (*Lactuca sativa* L.). Journal of Plant Nutrition, 2013; 36(11): 1659–1677.

[5] Zhang M K, Liu L J, Huang C. Effects of long-term irrigation of livestock farm wastewater on soil quality and vegetable quality in vegatable soils. Journal of Soil & Water Conservation, 2011; 25(1): 87–91.

[6] Szymanski N, Patterson R A. Effective microorganisms (EM) and wastewater systems. Future Directions for On-site Systems: Best Management Practice, 2003; pp.347–355.

[7] Zakaria Z, Gairola S, Shariff N M. Effective microorganisms (EM) technology for water quality restoration and potential for sustainable water resources and management. Biology Programme, School of Distance Education, 2010; 11800: 1–8.

[8] Yang L, Li T, Li F, Lemcoff J H, Cohen S. Fertilization regulates soil enzymatic activity and fertility dynamics in a cucumber field. Scientia Horticulturae, 2008; 116(1): 21–26.

[9] Roldán A, Salinas-García, J R, Alguacil M M, Caravaca F. Changes in soil enzyme activity, fertility, aggregation and c sequestration mediated by conservation tillage practices and water regime in a maize field. Applied Soil Ecology, 2005; 30(1): 11－20.

[10] Boelee E. Managing water and agroecosystems for food

security. CABI, Oxfordshire, UK, 2013.

[11] Morugán-Coronado A, García-Orenes F, Mataix-Solera J. Short-term effects of treated wastewater irrigation on Mediterranean calcareous soil. Soil & Tillage Research, 2011; 112(1): 18–26.

[12] Chen W, Wu L, Jr F W, et al. Soil enzyme activities of long-term reclaimed wastewater-irrigated soils. Journal of Environmental Quality, 2008; 37(5): 36–42.

[13] Bar-Tal A. Use of Treated Wastewater in Agriculture. Impacts on Soil, Environment and Crop. Willey-Blackwell, UK. pp. 31–165.

[14] Pedrero F, Kalavrouziotis I, Alarcón J J, Koukoulakis P, Asano T. Use of treated municipal wastewater in irrigated agriculture-Review of some practices in Spain and Greece. Agricultural Water Management, 2010; 97(9): 1233–1241.

[15] Adrover M, Farrús E, Moyà G. Chemical properties and biological activity in soils of Mallorca following twenty years of treated wastewater irrigation. Journal of Environmental Management, 2012; 95(95): 188–192.

[16] Chaperon S, Sauvé S. Toxicity interaction of metals (Ag, Cu, Hg, Zn) to urease and dehydrogenase activities in soils. Soil Biology & Biochemistry, 2007; 39(9): 2329–2338.

[17] Kaschuk G, Alberton O, Hungria M. Three decades of soil microbial biomass studies in Brazilian ecosystems: Lessons learned about soil quality and indications for improving sustainability. Soil Biology & Biochemistry, 2010; 42(1): 1–13.

[18] Shao X H, Liu D Y, Zhu L, Wang H. Prospect of EM treatment techniques of organic waste water for agricultural irrigation. Advances in Science and Technology of Water Resources, 2001; 21(1): 16–17.

[19] Mapanda F, Mangwayana E N, Nyamangara J, Giller K E. The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. Agriculture Ecosystems & Environment, 2005; 107(2): 151–165.

[20] Khan S, Lin A, Zhang S, Hu Q, Zhu Y G. Accumulation of polycyclic aromatic hydrocarbons and heavy metals in lettuce grown in the soils contaminated with long-term wastewater irrigation. Journal of Hazardous Materials, 2008; 152(2): 506–515.

[21] Wang J F, Wang G X, Wan Y H. Treated wastewater irrigation effect on soil, crop and environment: Wastewater recycling in the loess area of China. Journal of Environmental Sciences, 2007; 19(9): 1093–1099.

[22] Sun Y B, Sun G H, Zhou Q X, Xu Y M, Wang L, Liang X F, et al. Induced-phytoextraction of heavy metals from contaminated soil irrigated by industrial wastewater with Marvel of Peru (*Mirabilis jalapa* L.). Plant, Soil and Environment, 2011; 57(8): 364–371.

[23] Guan S Y. Soil Enzyme and Study Method. Beijing: China Agriculture Press, 1986. (In Chinese)

[24] Lu R K. Analysis methods of Soil Agricultural Chemistry. Beijing: China Agriculture Science and Technique Press, 2000. (in Chinese)

[25] Bowles T M, Acosta-Martínez V, Calderón F. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. Soil Biology and Biochemistry, 2014; 68: 252–262.

[26] Jalali M, Merikhpour H, Kaledhonkar M J, Van Der Zee S E A T M. Effects of wastewater irrigation on soil sodicity and nutrient leaching in calcareous soils. Agricultural water management, 2008; 95(2): 143–153.

[27] Dimitriou I, Aronsson P. Nitrogen leaching from short-rotation willow coppice after intensive irrigation with wastewater. Biomass and Bioenergy, 2004; 26(5): 433–441.

[28] Rusan M J M, Hinnawi S, Rousan L. Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. Desalination, 2007; 215(1): 143–152.

[29] Kou Y P, Wei K, Chen G X, Wang Z Y, Xu H. Effects of 3,4-dimethylpyrazole phosphate and dicyandiamide on nitrous oxide emission in a greenhouse vegetable soil. Plant, Soil and Environment, 2015; 61(1): 29–35.

[30] Liang Q, Gao R, Xi B, Zhang Y, Zhang H. Long-term effects of irrigation using water from the river receiving treated industrial wastewater on soil organic carbon fractions and enzyme activities. Agricultural Water Management, 2014; 135: 100–108.

[31] Minhas P S, Yadav R K. Long-term impact of wastewater irrigation and nutrient rates II. Nutrient balance, nitrate leaching and soil properties under peri-urban cropping systems. Agricultural Water Management, 2015; 156: 110–117.

[32] Hu X F, Jiang Y, Shu Y. Effects of mining wastewater discharges on heavy metal pollution and soil enzyme activity of the paddy fields. Journal of Geochemical Exploration, 2014; 147: 139–150.

[33] He J, Mark D, Arriaga J F, Fulton P J, Wood W, Shaw J N, et al. Short-term soil nutrient impact in a real-time drain field soil moisture–controlled SDI wastewater disposal system. Irrigation Science, 2013; 31(1): 59–67.

[34] Sun B Y. Effects of continuous cropping of flue-cured tobacco on the physical and chemical properties and urease activity at the arable layer of soil. Journal of Anhui Agricultural Sciences, 2010; 38(4): 1826–1827.

[35] Heinze S, Chen Y, El-Nahhal Y, Jung R, Safi J, et al. Small scale stratification of microbial activity parameters in Mediterranean soils under freshwater and treated wastewater irrigation. Soil Biology and Biochemistry, 2014; 70: 193–204.

[36] Krämer S, Green D M. Acid and alkaline phosphatase dynamics and their relationship to soil microclimate in a semiarid woodland. Soil Biology and Biochemistry, 2000; 32(2): 179–188.

[37] Kayikcioglu H H. Short-term effects of irrigation with treated domestic wastewater on microbiological activity of a Vertic xerofluvent soil under Mediterranean conditions. Journal of Environmental Management, 2012; 102: 108–114.

[38] Shi Z J, Lu Y, Xu Z G, Fu S L. Enzyme activities of urban soils under different land use in the Shenzhen city, China. Plant, Soil and Environment, 2008; 54(8): 341–346.

[39] Tao B X, Zhang J C, Cui Z H, Kong Y G, Yu Y C. Soil enzyme activity under different forest stands and its correlation with soil physical and chemical characters in the south hilly region of Jiangsu Province. Journal of Ecology and Rural Environment, 2009; 25(2): 44–48.

[40] Ma S C, Zhang H B, Ma S T, Wang G X, Shao Y, et al. Effects of mine wastewater irrigation on activities of soil enzymes and physiological properties, heavy metal uptake and grain yield in winter wheat. Ecotoxicology and Environmental Safety, 2015; 113(113C): 483–490.