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Influence of Metal-Enriched Sewage Sludge on Soil Rhizosphere Microbial Community of Winter Wheat (*Triticum aestivum* L.)

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A greenhouse pot experiment was conducted to evaluate the effect of sewage sludge enriched with Cd, Cu, Zn and Hg on soil rhizosphere microbial community of winter wheat (*Triticum aestivum* L.). The results showed that wheat biomass was negatively influenced by adding sewage sludge and heavy metals, microbial biomass carbon (C_{mic}), functional diversity (H') and average well color development (AWCD) significantly increased with increasing the application rate of sewage sludge. The C_{mic} and the ratio of C_{mic} and organic carbon (C_{org}) obviously increased with adding the contents of heavy metals at without sludge treatments, while the wheat biomass obviously decreased. Furthermore, there was the greater negative effect of combination between sewage sludge and heavy metals on microbial properties. Our study suggested that it was necessary to apply sewage sludge on a small scale and lay special stress on the importance of removing the heavy metals from sewage sludge to facilitate the long-term agricultural use in croplands.

Key words: Sewage sludge, Heavy metal, Microbial community, Rhizosphere soil, Wheat.

As one of the important waste disposal alternatives, land application of sewage sludge (SS) has gained worldwide acceptance. Being rich in organic matter, a valuable source of plant nutrients and also reducing significantly the sludge disposal cost component of sewage treatment, SS has been treated as a fertilizer which helps to increase crop production[1, 2]. However, there are heavy metals (HM) in SS which accumulate in soil with the long-term application, this can give rise to potential risks to soil microbial communities and their processes that are fundamental to maintaining soil health, ecosystem function and element cycle[3, 4]. Furthermore, SS also significantly reduced plant growth and development[5, 6]. Although, the potential impacts of heavy metals in SS on soil microbial communities and processes has been studies, it is still unable to draw clear conclusions about these effects due to the presence of multiple heavy metals[7, 8] and the variation of the microbial response to these metals, especially for some SS from specific regions[9, 10]. Moreover, microorganisms in the rhizosphere have a specific physicochemical environment and is further influenced by plants[11], which is also the important field in land application of SS. In addition, soil microbial assays have often been used to assess the effect of heavy metals (HM) contamination on long-term usage of SS[12, 13].

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In China, the amount of SS increases annually due to the strict demand for water quality and the rising number of wastewater treatment plants. Approximately more than 1.3×10^6 t (dry weight) of municipal wastewater is produced annually and the increasing annually rate is over 10 %[14], and land application of sewage sludge is an important and promising recycling approach[14]. SS enriched with Cd, Cu, Zn and Hg in this study was collected from the Waste Water Treatment Plant of Jinan, Shandong Province, mainly originated from domestic and industrial effluent, and the concentrations of HM in sludge were below the national standard of China for agricultural use according to our previous research[15].

The present study was to evaluate the effects of sludge enriched with Cd, Cu, Zn and Hg on the biomass and soil plant rhizosphere microbial community of winter wheat (*Triticum aestivum* L.), to assess microbial community's response and ecological and environmental risk of the SS as the land application, and to know the long-term effects through additional inorganic metal salts to simulate the accumulation of HM with long-term use of SS. The results will facilitate the agricultural use of SS.

MATERIALS AND METHODS

Sampling and preparation of soils and sewage sludge

A bulk soil sample was collected at 0-20 cm depth from the experimental field in Shandong Academy of Agricultural Sciences, Jinan, China. SS was collected from the Waste Water Treatment Plant of Jinan, China. The soil and SS were separately ground, air-dried, mixed thoroughly and analyzed for basic properties. The samples were passed through a 2-mm sieve for soil particle size distribution, and a portion of soil samples were passed through 1-mm sieve for soil pH, and then 0.25-mm were used for sieving soil samples to determine cation exchange capacity (CEC), as well as total nitrogen (N_t), and finally a 0.125-mm sieve for soil organic matter (OM) and analyses of heavy metals.

Determination of soil properties

The determination of soil properties were performed as described previously[15]. In brief, soil pH was determined by a pH meter (PHS-3C, Leici, China) in a 1:2.5 soil/ water ratio suspension. Cation exchange capacity (CEC) was determined by EDTA-acetic ammonium saturation. Soil was digested by potassium dichromate-sulfuric acid to measure total nitrogen (N_t), and was digested by sulfuric acid-perchloric acid and then molybdenum-antimony colorimetry for the measurement of total phosphorus (P_t). Organic matter, and organic carbon were detected by oxidation with a potassium dichromate-titration of $FeSO_4$. Procedures for the determination of basic soil properties followed standard methods recommended by the Chinese Society of Soil Science[16]. Soil particle size distribution was measured by a micro-pipette method[17].

Total background Cu, Zn and Cd contents in the soil and SS were analyzed after acid digestion. After being digested with a mixture of concentrated HNO_3 and HC1 (1:3, v/v) by the AIM600 Block Digestion System (AIM 600, A.i.Scientific, Australia), Cu and Zn were determined by atomic absorption spectrometry (TAS-990, Beijing Purkinje General, China). Cd was determined by graphite furnace atomic absorption spectrometry (SOLAAR M6, Thermo Electron, USA). Hg was determined by atomic fluorescence spectrometry (AFS-930, Jitan, China) after digestion in the AIM600 Block Digestion System with teardrops using aqua fortis (1:1)[18]. The quality control of the analysis was achieved by standard solution and standard sample (National Research Center for Certified Reference Materials, China). Characteristics and heavy metals contents of the soil and SS are shown in Table 1.

Treatments

SS had previously been air-dried for about 1 month[19]. The soil was added by Cd, Cu, Zn and Hg in salt forms: $3CdSO_4\cdot8H_2O$, $CuSO_4\cdot5H_2O$ and $ZnSO_4\cdot7H_2O$ and $HgCl_2$. Based on the concentrations of HM of SS and study about SS application rate[1], seven treatments (CK: control, T1: soil + 20% SS (4:1, w/w), T2: soil + 40% SS (3:2, w/w), T3: soil + 20% SS (4:1, w/w) + 5 × HM, T4: soil + 20% SS (4:1, w/w) + 10 × HM, T5: soil + 6 × HM, T6: soil + 11 × HM) were used in this experiment. The final concentrations of total Cd, Cu, Zn and Hg in soil are shown in Table 2. Basal fertilizers, which were NH_4NO_3 (150 mg N $\cdot kg^{-1}$ soil) and KH_2PO_4 (150 mg P $\cdot kg^{-1}$ soil), were only applied to treatments without SS. Each treatment had four

replicates. Then the soils were transferred to pots with 2000 g soil per pot and pre-incubated at 25 °C for seven days. After conditioning, a rhizobox made of 48-ìm nylon screen was applied in each pot with 200 g soil.

Plant culture

The pot experiment was carried out in a greenhouse with temperature of 25 ± 3 °C in the daytime and 20 ± 3 °C at night. The seeds of winter wheat (*Triticum aestivum* L.) (Shannong 12) were disinfected in 10% H_2O_2 solution for 10 min followed by thorough washing in deionized water, then refrigerated at 0-4 °C for 24 h. Finally, seeds were germinated on moist filter paper for 2-3 days in an Artificial Climate Box (HPG-400H, Donglian, China) (Temperature: 25 ± 1 °C, humidity: 50%). Fifteen seeds were sown in each rhizobox. Ten days after germination, seedlings were thinned to ten uniform plants. The water content of the soil was watered up to 20%. Throughout the growth period, water losses were compensated for by the addition of deionized water. After 8 weeks of growth, plants were harvested. Rhizosphere soil and non-rhizosphere soil were collected separately. Soil was divided into two parts, one part was air-dried, the other part was sieved (2-mm mesh) and stored at 4 °C before the microbial test. Plants were washed with deionized water and dried with soft paper, fresh weights were determined immediately. Plant materials were then oven-dried at 70 °C for 72 h, and dry weights were determined for all tissues.

Soil microbial biomass and basal respiration

Basal respiration (BAS) and microbial biomass carbon (C_{mic}) were measured using the method described by Nikliñska *et al*.[20] with modification. For BAS measurements, 5 g (dry weight equivalent) of fresh soil samples were adjusted to 50 ± 5% of their water holding capacity, and then incubated at 25 ± 1 °C in gas-tight jars for 24 h. Small vessels with 5 ml 0.2 mol·L^{-1} NaOH in the jars absorbed the evolved CO_2, and added 2 ml $BaCl_2$ (0.5 mol·L^{-1}) to the NaOH after opening the jars, then the excess hydroxide was titrated with 0.1 mol·L^{-1} HCl in the presence of phenolphthalein as an indicator. A similar procedure was followed for the C_{mic} measurements, with the difference that fresh soil samples were amended with 0.02 g glucose monohydrate per gram soil for substrate-induced respiration (SIR) measurements, and then were incubated at 25 ± 1 °C in gas-tight jars for 4 h. C_{mic} was calculated from the SIR rate: C_{mic} (mg·g^{-1}) = 40.04 y + 0.037, where y is given in mL CO_2 ·h^{-1} · g^{-1}.

Biolog ECO plate analyses

Soil samples were serially diluted to a 10^{-3} suspension in sterile saline solution (0.85%, m/v) for inoculation to Biolog plates. Each well of an Eco-Biolog plate was inoculated with 150 ìL suspension using a multipipettor. The plates were inoculated at 25 ± 1 °C in the dark and then were scanned at 590 nm. The first two measurements were carried out at 20 h and 36 h after incubation, and the subsequent ones were determined at 12 h intervals for 5 days. The data was collected by Microlog™ Release 4.20 software (ML3402, Microlog, USA). The 72 h absorbance values were used for calculating diversity index and PCA[21].

Data analysis

For community level physiological profiling (CLPP) analysis, AWCD was calculated according to Garland and Mills[22].

$$\text{AWCD}=\sum(C-R)/n \qquad ...(1)$$

where *C* is color production within each well (optical density measurement), *R* is the absorbance value of the plates control well, and *n* is the number of substrates (Eco plates, *n*=31).

CLPP diversity was calculated as the Shannon index (H' and *E*), whereis Shannon's diversity index.

$$H' = -\sum P_i \ln P_i \text{ (i is from 1 to } S) \qquad ...(2)$$

P_i means proportional color development of the th well (*C*-*R*) over total color development of all wells of a plate, *S* means number of wells with color development (total data of species).

Species evenness was calculated as *E*.

$$E = H'/\ln S \qquad ....(3)$$

the calculation of H' and E was based on 72 h incubation readings[23].

PCA was used to characterize community level profiles. Data obtained by CLPP were interpreted by PCA using Statistic 6.0. The first two PCs (PC1 and PC2) were subsequently plotted to visualize the results. Plant results, AWCD and

diversity index were compared by ANOVA with SPSS 13.0.

RESULTS AND DISCUSSION

Biomass of wheat

The results showed that wheat biomass was negatively influenced by adding the amount of SS and HM (Fig. 1). During the growth period, the roots as a very sensitive parameter of wheat were harmed in sludge-amended soils, and may be poisoned by SS, which is in consistent with the results obtained by other researches[24]. The total weight of wheat with SS was much lower than that without SS. In fact, the biomass of wheat significantly decreased with increasing amounts of HM ($P< 0.05$). Therefore, this strong negative effect might have been resulted from other components of the SS besides HM, such as PAHs, PCDD/Fs and pathogenic bacteria present in the sewage sludge may also result in the harm to plant[25].

Chemical and microbial properties

Soil chemical and microbial characteristics under different amendments are shown in Table 3. The contents of N_t, C_{org} and microbial biomass (C_{mic}) significantly increased with the increasing amounts of SS in the soil ($P< 0.05$), while pH significantly decreased ($P< 0.05$). The decrease of pH was mainly resulted from the extreme low pH of the SS, and very low soil pH might be an important factor influencing the growth of most crops[6]. Hydrogen ions in the growth medium were also found generally inhibit root growth[26]. Basal respiration (BAS) also increased with increasing amounts of SS in the rhizosphere, while there was no difference among the treatments. However, BAS significantly increased with increasing amounts of SS in the non-rhizosphere ($P< 0.05$). Carbonell *et al.*[27] also found that the enzymatic activities (dehydrogenase and phosphatase) and soil basal respiration rate (as a parameter used to monitor microbial activity), were increased in sewage-amended soils. The change of C_{org} / N_t was mainly resulted from the sludge. Additionally, with increasing contents of HM in the soil, the values

Table 1. Basic properties of soil and sewage sludge used in this study [§)]

Properties	Soil	Sewage sludge
pH/ (H_2O)	8.06 (0.01)	6.55 (0.02)
particle size distribution/ (%)		
sand (0.05-2mm)	11.31	-
silt (0.002-0.05mm)	78.57	-
clay (<0.002mm)	10.12	-
textural classification	Silt loam	-
cation exchange capacity/ ($cmol \cdot kg^{-1}$)	15.5 (0.05)	-
organic matter/ (%)	1.47 (0.05)	37.01 (1.91)
total N/ (%)	0.08 (0.0004)	2.68 (0.05)
total P/ (%)	0.04 (0.001)	0.33 (0.01)
total Cd/ ($mg \cdot kg^{-1}$)	0.08 (0.01)	0.99 (0.07)
total Cu/ ($mg \cdot kg^{-1}$)	30.86 (1.82)	153.8 (0.40)
total Zn/ ($mg \cdot kg^{-1}$)	79.37 (1.08)	716.3 (8.20)
total Hg/ ($mg \cdot kg^{-1}$)	0.55 (0.34)	12.16 (0.53)

Note: [§)] average of three samples (standard deviation)

Table 2. Concentrations of total Cd, Cu, Zn and Hg in soil after amendment [§)]

Treatments	Heavy metals/ ($mg \cdot kg^{-1}$)			
	Cd	Cu	Zn	Hg
Ck	0.08	30.86	79.37	0.55
t1	0.278	61.62	222.63	2.98
t2	0.476	92.38	365.89	5.41
t3	1.27	215.42	938.93	15.14
t4	2.26	369.22	1655.23	27.3
t5	1.27	215.42	938.93	15.14
t6	2.26	369.22	1655.23	27.3

Note: [§)] ck: control, t1: soil + 20% SS (4:1, w/w), t2: soil + 40% SS (3:2, w/w), t3: soil + 20% SS (4:1, w/w) + 5 × HM, t4: soil + 20% SS (4:1, w/w) + 10 × HM, t5: soil + 6 × HM, t6: soil + 11 × HM

of C_{mic} and C_{mic} / C_{org} significantly increased in treatments without sludge ($P< 0.05$). For sludge-heavy metal treated soils, the value of BAS and C_{mic} increased compared with control, while C_{mic} / C_{org}, C_{org} / N_t significantly decreased. Therefore, the application of SS and HM together to soil had greater effects to C_{mic} and C_{org} / $N_t$than that of the sludge or HM alone.

It is usually considered that large contents of organic matter, N_t and P_t in SS were able to amend soil qualities, while accumulated HM in SS can result in the decrease of microbial biomass andC_{mic} / C_{org} [28]. Although there was an increase in BAS and C_{mic}, and a decrease in C_{org} / N_t and C_{mic} / C_{org} with increasing amounts of SS in the soil, an additional effect due to the increasing contents of HM in the soil was also observed. The reason for this enhancement to BAS and C_{mic} was the easily degradable organic matter in SS, which induced an increase of the short term mineralization process in the soil [29]. It was also found that the use of sludge with higher metal concentrations may lead to short-term changes in soil microbial communities and their activities [3], with increased loss of C to the atmosphere and N availability. Montserrat *et al.*[30] also found the similar results at the high SS application rates. However, the decrease in C_{mic} / C_{org} and C_{org} / N_t meant that the increasing rate of C_{org} was higher than that of C_{mic}, and the increasing rate of N_t was higher than that of C_{org}. As shown, short-term SS application increased C_{org} and N_t of soil, however, the

Table 3. Soil chemical and microbial characteristics at different treatments [§)]

Treatments		pH/ (H_2O)	N_t/ (%)	C_{org}/ (%)	C_{org} / N_t	C_{mic}	BAS	C_{mic} / C_{org}
r	Ck	8.12 (0.01) d	0.07 (0.003) a	0.81 (0.02) a	11.68 (0.5) b	0.94 (0.11) a	7.41 (2.49) a	0.85 (0.29) a
	t1	6.96 (0.03) c	0.52 (0.01) b	3.92 (0.14) b	7.55 (0.18) a	2.46 (0.31) bc	13.38 (2.35) a	0.63 (0.08) a
	t2	6.59 (0.02) b	0.84 (0.01) d	7.08 (0.10) c	8.41 (0.11) a	4.33 (0.29) d	17.25 (2.98) a	0.61 (0.05) a
	t3	6.61 (0.01) b	0.56 (0.005) bc	4.14 (0.12) b	7.45 (0.26) a	1.63 (0.28) ab	8.67 (4.55) a	0.39 (0.05) a
	t4	6.44 (0.04) a	0.57 (0.03) c	4.27 (0.20) b	7.52 (0.25) a	1.66 (0.18) ab	11.19 (1.57) a	0.39 (0.06) a
	t5	7.69 (0.01) d	0.07 (0.002) a	0.79 (0.01) a	10.94 (0.48) b	2.37 (0.29) bc	7.92 (0.86) a	2.98 (0.29) b
	t6	7.58 (0.02) d	0.07 (0.002) a	0.72 (0.01) a	10.52 (0.32) b	2.90 (0.15) c	10.09 (0.58) a	4.01 (0.20) c
nr	Ck	8.12 (0.004) f	0.07 (0.003) a	0.74 (0.01) a	10.9 (0.48) b	0.98 (0.08) a	16.64 (2.38) b	1.31 (0.1) b
	t1	7.03 (0.03) c	0.47 (0.02) b	3.6 (0.07) b	7.67 (0.23) a	1.73 (0.05) a	20.93 (1.26) bc	0.47 (0.02) a
	t2	6.63 (0.03) b	0.88 (0.03) d	7.03 (0.07) d	7.96 (0.17) a	2.55 (0.16) b	29.75 (2.78) d	0.36 (0.03) a
	t3	6.72 (0.02) b	0.53 (0.01) bc	3.97 (0.19) b	7.56 (0.32) a	1.12 (0.19) a	26.39 (1.62) cd	0.28 (0.05) a
	t4	6.47 (0.03) a	0.55 (0.01) c	4.42 (0.10) c	8.11 (0.22) a	1.32 (0.23) a	8.32 (0.86) a	0.3 (0.05) a
	t5	7.96 (0.02) e	0.07 (0.003) a	0.76 (0.01) a	10.99 (0.72) b	1.37 (0.14) a	3.38 (0.63)a	1.8 (0.16) b
	t6	7.75 (0.04) d	0.07 (0.004) a	0.68 (0.01) a	9.13 (0.41) ab	2.55 (0.13) b	6.86 (1.08) a	3.76 (0.21) c

Note: [§)] Average of four replicates (standard deviation). The same letters in each column are not significantly different. Tukey HSD test, $P < 0.05$. ck: control, t1: soil + 20% SS (4:1, w/w), t2: soil + 40% SS (3:2, w/w), t3: soil + 20% SS (4:1, w/w) + 5 × HM, t4: soil + 20% SS (4:1, w/w) + 10 × HM, t5: soil + 6 × HM, t6: soil + 11 × HM. N_t: total nitrogen; C_{org}: organic carbon; C_{mic}: microbial biomass carbon; BAS: Basal respiration; r: rhizosphere; nr: non-rhizosphere.

cooperative effects of the large quantity of SS application and high concentrations of HM resulted in the decrease of pH, C_{org} / N_t and C_{mic} / C_{org}. Therefore, a lower application rate of sewage sludge for agriculture soil by weight would have an optimal effect on soil microbial activity and accumulation of heavy metals.

CLPP analysis of soil microbial communities

The value of AWCD of each treatment incubation time was calculated and graphed (Fig. 2). The results showed that the values of AWCD significantly decreased with the increasing amounts of SS and HM ($P < 0.05$). The sludge-amended treatments had a slow rate of color development on BIOLOG plates, which was in agreement with Kelly *et al*.[12]. Gomes *et al*.[4] also showed that highly metal- contaminated sludge (Cd- and Zn- enriched sewage sludge) application may affect the soil microbial communities through increased inputs of potential heavy metals, and particularly have both short- and long-term effects on various bacterial phylogenetic groups. Furthermore, there was no significant difference in the values of AWCD between the rhizosphere and non-rhizosphere with high application rates of SS (40%) and HM (11 times).

The first two principal components (PC) of the CLPP data explained 47.01% of total variance with PC1 accounted for 30.66%, which are shown in Fig. 3 (a). Biolog substrates loadings contributing to the community ordination pattern are shown in Fig. 3 (b). In the rhizosphere, the treatments without SS tended to have greater utilization of the carbon sources found to the right of the origin, whereas the treatments with SS (20% SS and 20% SS + 5 × HM) made use of the carbon

Table 4. Diversity and evenness indices for the treatments [§)]

Treatments	H'	E	H'	E
	Rhizosphere		Non-rhizosphere	
ck	2.98 (0.02) [§] b[£]	0.98 (0.004) a	2.55 (0.09) a	1.04 (0.04) a
t1	2.83 (0.06) ab	1.12 (0.09) a	2.72 (0.11) ab	1.29 (0.15) a
t2	2.89 (0.09) bc	1.18 (0.07) a	2.94 (0.03) b	1.29 (0.12) a
t3	2.69 (0.08) a	1.04 (0.05) a	2.86 (0.11) ab	1.38 (0.19) a
t4	2.83 (0.08) ab	1.17 (0.08) a	3.00 (0.02) b	1.27 (0.15) a
t5	2.87 (0.02) ab	1.00 (0.01) a	2.53 (0.08) a	1.28 (0.18) a
t6	2.86 (0.05) ab	1.01 (0.02) a	2.72 (0.05) ab	0.98 (0.02) a

Note: [§)] Average of four replicates (standard deviation). [£]The same letters in each column are not significantly different. Tukey HSD test, $P < 0.05$. ck: control, t1: soil + 20% SS (4:1, w/w), t2: soil + 40% SS(3:2, w/w), t3: soil + 20% SS (4:1, w/w)+ 5 × HM, t4: soil + 20% SS (4:1, w/w)+ 10 × HM, t5: soil + 6 × HM, t6: soil + 11 × HM. : Shannon's diversity index; *E*: Species evenness index.

sources mainly found beneath the origin. The carbon sources found on the left of the origin were used by the treatments with high application rates of SS and HM (40% SS and 20% SS + 10 × HM). The treatments tended to have higher utilization of the carbon sources in the rhizosphere than that in the non-rhizosphere. However, the high application rate of SS (40% SS), the high addition rate of HM (11 × HM) and the application of SS with high addition rate of HM (20% SS + 10 × HM) had the similar utilization ability of the carbon sources between the rhizosphere and non-rhizosphere. Furthermore, the treatments without SS had higher utilization of most of the substrates than that of other treatments. That is to say, the application of SS decreased the utilization ability of the carbon sources of microbial communities in the soil. On the other hand, it is usually considered that the microorganisms in the rhizosphere have a higher activity compared with that in the non-rhizosphere[31, 32], while the high application rate of SS and the high addition rate of HM in this study reduced the difference between rhizosphere and non-rhizosphere from the Biolog test.

Functional diversity of soil microorganisms

Shannon index (H') and evenness (E) calculated using the 72 h data are listed in Table 4. The results showed that the control had the highest value of Shannon index. In the rhizosphere, the value of decreased withincreasing amounts of

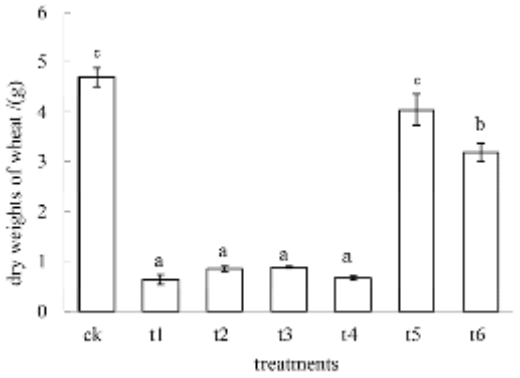

Fig. 1. Dry weights of wheat (The same letters in each column are not significantly different. Tukey HSD test, $P < 0.05$)

ck: control, t1: soil + 20% SS (4:1, w/w), t2: soil + 40% SS (3:2, w/w), t3: soil + 20% SS (4:1, w/w) + 5 × HM, t4: soil + 20% SS (4:1, w/w) + 10 × HM, t5: soil + 6 × HM, t6: soil + 11 × HM.

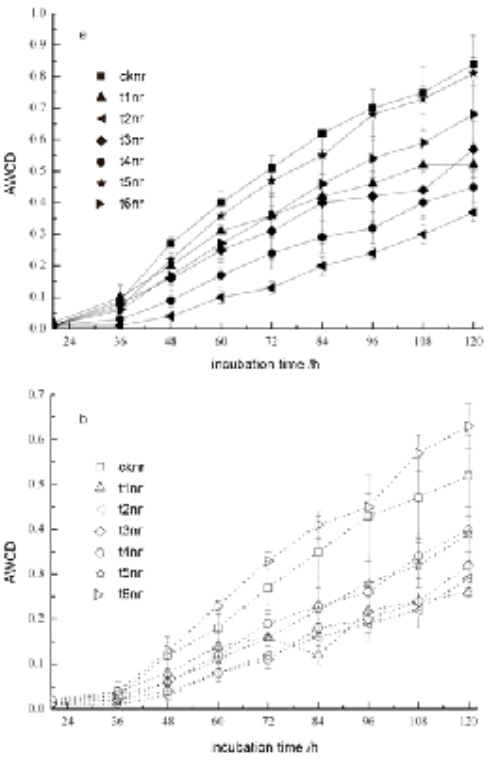

Fig. 2. Average well color development (AWCD) of each soil treatment: Rhizosphere (A) and non-rhizosphere (B) in growth periods.

ck: control, t1: soil + 20% SS (4:1, w/w), t2: soil + 40% SS (3:2, w/w), t3: soil + 20% SS (4:1, w/w) + 5 × HM, t4: soil + 20% SS (4:1, w/w) + 10 × HM, t5: soil + 6 × HM, t6: soil + 11 × HM

SS and HM. The treatments of SS and HM together had greater negative effects to the value of than that of the HM alone. On the contrary, the value of increased with increasing amounts of SS and HM in the non-rhizosphere. The HM alonehad greater negative effects to the value of than that of the treatments of SS and HM together. However, thedid not show the difference among all of the treatments. Furthermore, the value of in the rhizosphere was much higher than that of the bulk soil at the control treatment.

Soil microbial function diversity from CLPP data may be used in monitoring changes of microbial diversity caused by environmental fluctuations and pollution[23]. There are many factors influencing microbial diversity, such as

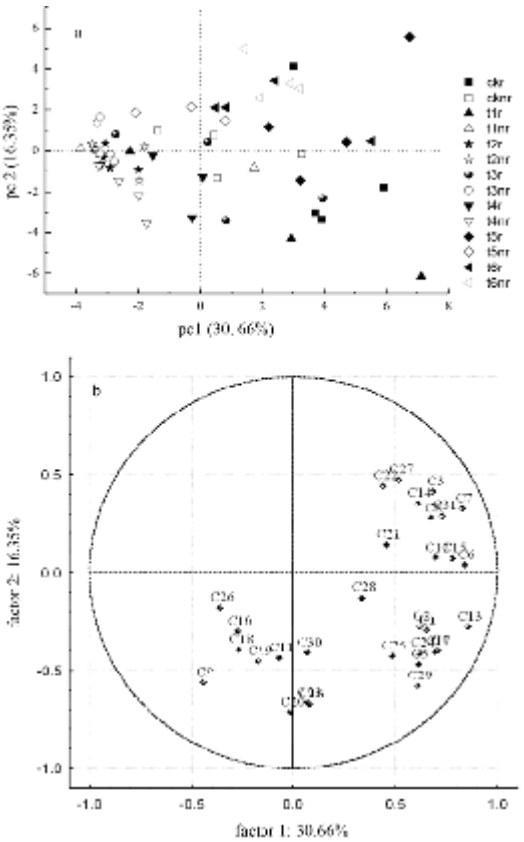

Fig. 3. Principal components analysis (PCA) of Eco-Biolog plates profiles from microbial communities of soil treatments (a). Loadings of Biolog substrates in PCA (b). r: rhizosphere, nr: non-rhizosphere.

ck: control, t1: soil + 20% SS (4:1, w/w), t2: soil + 40% SS (3:2, w/w), t3: soil + 20% SS (4:1, w/w) + 5 × HM, t4: soil + 20% SS (4:1, w/w) + 10 × HM, t5: soil + 6 × HM, t6: soil + 11 × HM.

nutrient quality, soil texture and chemical pollution of soil[33]. For the accumulation of the HM with SS application, although Moffett *et al*.[34] found that Zn-contaminated soil (400 $mg \cdot kg^{-1}$) had lower bacterial diversity than that of control (57 mg Zn $\cdot kg^{-1}$), changes resulted from SS such as pH, C_{org} and N_t further influenced bacterial diversity in this study. Khan and Scullion[3] also confirmed that microbial populations and processes in the soil can be influenced by metals in sludge. In one word, soil slujdge applications may cause a wide range of modifications in soil composition, texture, biological activity and in some cases the presence of toxic micropollutants[27].

CONCLUSIONS

The results of simulating the accumulation of HM with long-term use of SS showed that wheat biomass was negatively influenced by adding the amount of sewage sludge and heavy metals. SS application increased C_{org} and N_t in soil, whereas large quantity of SS application and high concentration of HM decreased the substrates utilization ability of soil microorganisms, Shannon index, pH, C_{org} / N_t and C_{mic} / C_{org}. The results also showed the use of SS and HM together had greater negative effects to C_{org} / N_t, C_{mic} / C_{org} and AWCD than that of SS and HM alone. Furthermore, there was no significant difference on microbial properties between the rhizosphere and non-rhizosphere with high application rate of SS and the high addition rate of HM. It can be concluded that the negative effects of SS land application to microbial community will gradually increase with the accumulation of HM. As a result, it is necessary to have a low quantity application rate and remove the HM from the SS for long-term land application.

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