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Effects of Pesticide-Contamination on Population and Activity of Bacteria in Purple Paddy Soil

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Abstract

The effect of pesticides on microbial in soil is susally used as an important guide line for estimating the ecological safty of soil. Effects of pesticide-contamination on population and activity of bacteria were investigated in purple paddy soil by in door culture method. The results showed that the population and activity of methanogens was stimulated slightly by adding carbofuran or butachlor in paddy soil, but significantly inhibited by adding of butachlor (10 mg kg^{-1}), carbofuran (0.5 mg kg^{-1}) and carbendazim (10 mg kg^{-1}) in paddy soil. At 7th day the inhibition reached the maximum and then gradually reduced down to the level in control. 1 mg kg^{-1} carbufuran and butachlor markedly stimulate the populations and activity of soil methane-producing bacteria, soil anti-nitrifying bacteria, soil nitrogen-fixing bacteria, and soil sulfate-reducing bacteria in purple paddy rice soils. Higher concentration carbufuran and butachlor significantly inhibite the populations and activity of above bacteria. Then the inhibition effects of the contaminants were gradually attenuating. The population and activity of above bacteria were inhibited by different level carbendazim.

Key words: Purple paddy soil; Pesticide; Activity

1. Introduction

Extensive use of pesticides affected soil ecological environment and changed soil physical properties, and it also affected the populations and biological activity of soil microbial directly, thereby affecting

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their degradation capability of pesticide. In recent years, the relationship between chemicals and soil microbial has become an important research area of environmental science [1]. Therefore, the study of the impact of pesticides on soil microbial has become an important indicator to evaluate pesticides' effect on soil ecological security[2]. The studies of impact of pesticides contamination on soil microbial were mainly focused on dry land, woodland, grassland and mining areas, and systematic research of its impact on paddy soil anaerobic microbial populations and activity was rarely reported at home and abroad. Some anaerobic microbial with special physiological function in paddy soil, such as methane-producing bacteria, anaerobic nitrogen-fixing bacteria, denitrifying bacteria, sulfate-reducing bacteria and etc., played an important role in organic carbon decomposition, nitrogen cycling and greenhouse gases generating[3, 4]. Purple paddy soil was the material in this study, and indoor culture testing methods was taken. The impact of pesticide contamination on purple paddy soil microbial populations and biological activity was researched, and the feasible impact of different pesticides on biological properties of purple paddy soil was explored, in order to provide scientific basis for the safe and rational use of pesticides, pollution prevention and evaluation of pesticides' effect on soil ecological security.

2. Materials and methods

2.1. Tested Soil

Soil samples were collected from purple paddy soil cultivation layer (0 ~ 20 cm) in National Purple Soil Fertility and Fertilizer Effective Long-term Location Experiment Farm, Southwest University. Physical and chemical properties of the tested soils were as follows: content of soil organic matter 32.1 g kg^{-1}, Total N 1.52 g kg^{-1}, Available N, Available P, Available K were 83.1 mg kg^{-1}, 4.3 mg kg^{-1}, and 88.2 mg kg^{-1}, and pH of 7.27.

Visible gravels were removed from sample soil and organic residues remained. Certain copies of soil (equivalent to 1000 g dry soil) were placed in 1300 ml clean plastic box, and water was added to keep the soil waterlogged, and it was pre-cultured in 28 ℃ (constant temperature)for 3 weeks, in the purpose of microbial rejuvenation, especially for anaerobic microbial. It was pre-cultured for 3 weeks for post-pesticide treatment.

2.2. Experimental Design

Several boxes of soil which was pre-cultured for 2 weeks were added different concentrations (mg kg^{-1}) of carbofuran (0, 1, 10, 50), carbendazim (0, 0.5, 5, 50) and butachlor (0, 1, 10, 50), the process was repeated 20 times, and soil samples were mixed sufficiently at 28 ℃. After 0, 7, 14, 21, 28 days of culture, the samples (3 boxes) were tested for the population of microbial communities and their activity. Throughout the culture period, water was added momentarily, so the water level remained above the soil 1 ~ 2 cm, simulating the anaerobic environment of paddy field.

2.3. Determination and counting of microbial communities

Anaerobic microbial were prepared their mediums under anaerobic conditions, counted by roll tube count or MPN count method according to Heng Gete (Hungat) anaerobic technology[5,6].

Cultured at 28 ℃, denitrifying bacteria and anaerobic nitrogen-fixing bacteria were cultured for 7 days; methanogenic bacteria 30 days; sulfate-reducing bacteria 2 ~ 3 days. Denitrifying bacteria was counted by the bubble in Du-canaliculus; the growth indicator of methanogenic bacteria was the visible turbidity, supplemented by 102G gas chromatograph testing H_2, CH_4, counted by MPN; anaerobic nitrogen-fixing bacteria was counted directly by vitro colony[5]; sulfate-reducing bacteria was counted by the amount of black colonies in anaerobic tube[8].

2.4. Determination of paddy soil microbial activity

(1) Measurement and counting of paddy soil methanogenic activity were referred to in literature[7].
(2) Measurement and counting of paddy soil sulfate-reducing activity were referred to in literature[8].
(3) Determination and counting of denitrification activity were were referred to in literature [8].
(4) Measurement and counting of paddy soil nitrogenase activity were referred to in literature [4].

2.5. Data Analysis

All test data were the averages of three repeated tests, and data analysis were processed by SPSS 18.0 for windows software.

3. Results and Discuss

3.1. Impact of pesticide contamination on the amount of methane-producing bacteria and its activity

Fig. 1. indicated that in the first two weeks, amount of methane-producing bacteria was stimulated if 1 mg carbofuran or butachlor was added into 1kg paddy soil, in the passage of time, the simulation effect became weaker and recovered to the control level. When carbofuran or butachlor increased to 10 mg kg^{-1}, methane-producing activity was seriously inhibited, and the higher the concentration was, the stronger the inhibition was. Carbendazim had a serious inhibition impact on methane-producing bacteria, and the higher the concentration was, the stronger the inhibition was. Within two weeks, the inhibition rate of 0.5mg kg^{-1} carbendazim of soil methane-producing bacteria was 42%. Methane-producing bacteria was severely inhibited by 5mg kg^{-1} and 50mg kg^{-1} carbendazim, which was 54% and 85% lower than the control. In the 28th days of pesticides, methane-producing bacteria were still severely inhibited by 5mg kg^{-1} and 50mg kg^{-1} carbendazim.

Methanogenic activity was most sensitive to carbendazim. In the 7th day, methanogenic activities with the application of 0.5 mg kg^{-1}, 5 mg kg^{-1} and 50 mg kg^{-1} carbendazim were 50%、63% and 93% lower than the control, in the 28th day, serious depression remained (Fig. 2.). The result was similar to the conclusions of Chen and others' research on ponderosa paddy soil[10]. It indicated that the impact of the three pesticides on the amount of purple paddy methanogenic bacteria and its activity was similar to that on ponderosa paddy soil methanogenic bacteria. Methanogenic bacteria was the last of organic degradation and transforming chain in anaerobic conditions, and its amount and activity was representative in paddy soil. Other studies have shown that methane-producing bacteria was a microbial population sensitive to foreign pollution[11]. Whether the methanogenic bacteria activity can be a sensitive indicator of paddy soil pesticide contamination needed more tests to verify.

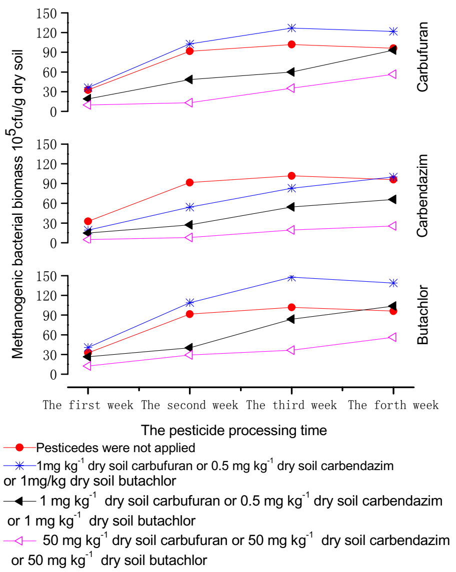

Fig. 1. Effect of pesticide condemnation the amount of methanogenic bacteria in the purple paddy soil

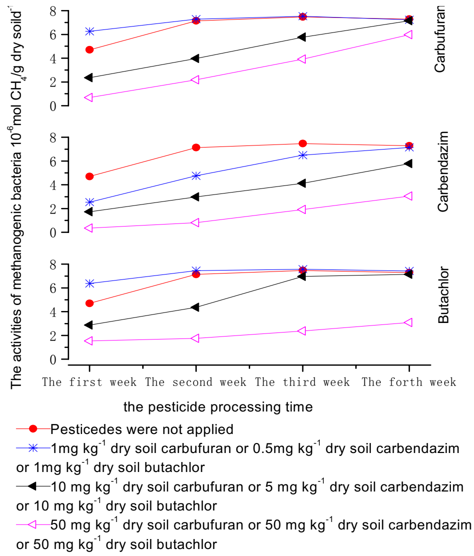

Fig. 2. Effect of pesticide pollution on the activities of methanogenic bacteria in the purple paddy soil

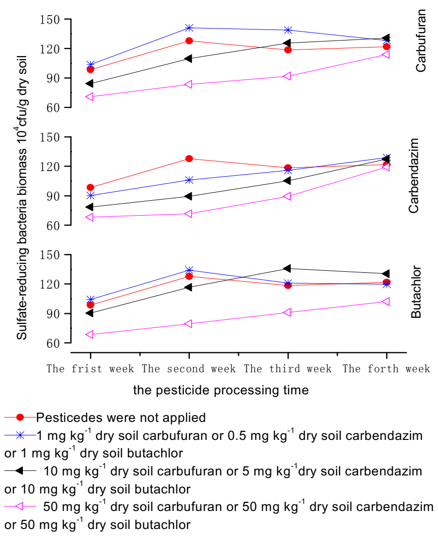

Fig. 3. Effect of pesticide pollution on the amount of sulfate-reducing bacteria in the purple paddy soil

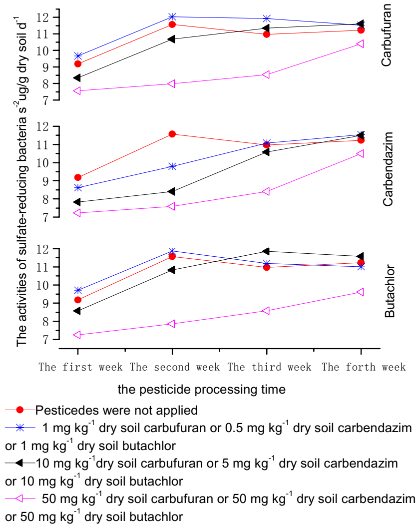

Fig. 4. Effect of pesticide pollution on the activities of sulfate reducing bacteria in the purple paddy soil

3.2. Impact of pesticide contamination on the amount of sulfate-reducing bacteria and its activity

Fig. 3. showed that 1 mg kg^{-1} carbofuran and butachlor can stimulate the growth of sulfate-reducing bacteria in purple paddy soil. High concentration inhibited the growth significantly, but with the passage of time, the inhibition effect gradually weakened. At all the concentrations of carbendazim treatment, the

growth of sulfate-reducing bacteria was inhibited, indicating that it was more sensitive to carbendazim (Fig. 4).

Application of different pesticides had great impact on the amount of sulfate-reducing bacteria and its activity in purple paddy soil. Chen and others' experiments also showed that low concentrations of carbofuran or butachlor can stimulate the growth of sulfate-reducing bacteria and its activity, but higher concentrations of butachlor or carbofuran inhibited its growth and activity, with time went by, inhibition gradually reduced. Different pesticides had different impact on different paddy soil. The amount and activity of sulfate-reducing bacteria of paddy soil of good water holding capacity were higher than that of paddy soil of poor water holding capacity[12].

3.3. Impact of pesticide contamination on the amount of denitrifying bacteria and its activity

Fig. 5. displayed the impact of pesticides over the quantity of soil denitrifying bacteria. Low-concentration of carbofuran or butachlor stimulated the growth of anti-nitrifying bacteria in purple paddy soil, while high-concentration inhibited the growth. With the passage of time, the inhibition would become weaker and almost disappeared at the 28th day. Denitrifying activity would be stimulated if 1mg carbofuran was added into 1kg paddy soil. 7 days later, anti-nitrifying activity was 5% higher than the control, and it was always higher than the control during the 28 days (Fig. 6.). When the concentration of carbofuran increased, the denitrification activity was first inhibited, with the passage of time, the inhibition gradually eliminated and converted to stimulating effect.

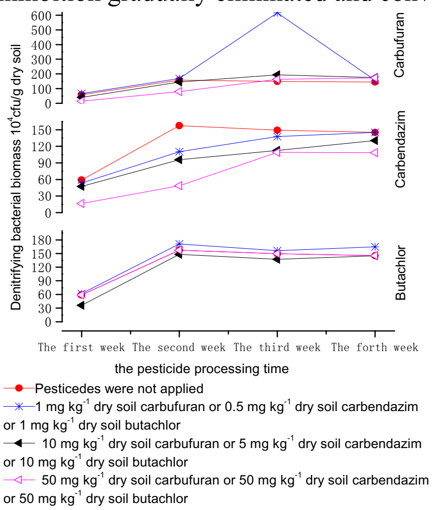

Fig. 5. Effect of pesticide pollution on the amount of denitrifying bacteria in the purple paddy soil

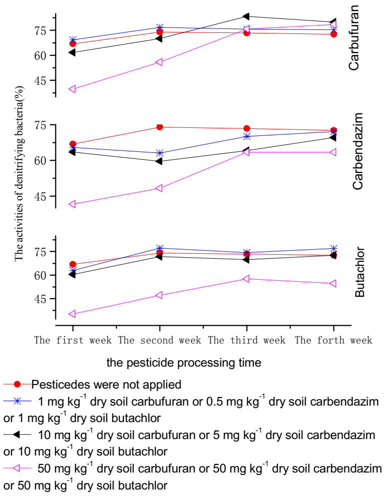

Fig. 6. Effect of pesticide pollution on the activities of denitrifying bacteria in the purple paddy soil

4. Conclusion

1)1 mg kg^{-1} of carbofuran or butachlor can stimulate the amount and activities of methanogenic bacteria, sulfate-reducing bacteria, denitrifying bacteria and anaerobic nitrogen-fixing bacteri in the initial stage of experiment; when 1 kg dry soil was added 5 mg carbendazim, 50 mg carbofuran or butachlor, the growth and activities of the microbial were inhibited significantly, the higher the concentration, the greater the inhibition, and the inhibition impact reached maximum in the 14th day.

2)Anaerobic nitrogen-fixing activity was more sensitive to pesticides than denitrifying activity and sulfate-deoxidizing activity. If 0.5 mg carbendazim was added in 1 kg paddy soil, anaerobic nitrogen-fixing activity would be inhibited. 7 days later, the anaerobic nitrogen-fixing activity dealt with carbendazim was 9% lower than that the control. And this inhibition was not reduced as time went by. When carbendazim increased as 5 mg kg^{-1} and 50 mg kg^{-1}, anaerobic nitrogen-fixing activity was seriously inhibited, and it was 34% and 57% less than the control at the 7th day. The inhibition would reduce with the passage of time and its activity gradually returned to control level.

Acknowledgements

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