Characteristics of chlorpyrifos removal and microbial community analysis for constructed wetlands systems

Jing Zhao¹, Weihong Zhua ², Yanji Wang ², Mingji Jin³,*

¹ Sciences College of Yanbian University, Yanji, Jilin 133002, China
² Agricultural College of Yanbian University, Yanji Jilin 133002, China
³ Joint Key Laboratory of Wentland & Ecology of Changbai Mountain Jilin Province, Yanji 133002, China

*Corresponding author E-mail: jinmingji@ybu.edu.cn

Abstract. The mechanisms of chlorpyrifos removal from constructed wetland systems were determined using different initial concentrations of chlorpyrifos. The microbial community structure in the wetland system was analyzed using high-throughput sequencing technology. The results showed that, in the wetland system, the removal of chlorpyrifos was accomplished by combined substrate adsorption, plant absorption, and microbial degradation. However, there were significant differences in the removal of chlorpyrifos by substrate, plants, and microbes when different initial concentrations of chlorpyrifos were used, and with different wetland systems. High-throughput sequencing results showed that there was a negative correlation between the microbial community richness in late operation of wetland systems and the initial concentration of chlorpyrifos. In the wetland systems, Proteobacteria and Acidobacteria were the predominant bacterial phyla that conducted the removal of chlorpyrifos.

Keywords: Constructed wetland; Chlorpyrifos; Removal characteristics; Microbial community; High-throughput sequencing.

1. Introduction

Chlorpyrifos is an organophosphorus pesticide of moderate toxicity and efficiency, and is mainly used for pest control in agriculture [1-2]. Presently, the inappropriate and excessive use of chlorpyrifos, pose a substantial threat to the environment [3]. Constructed wetland is an ecological treatment technology used to remove pollutants by using substrates, plants, and microbes. Constructed wetland has the advantages of low operation cost, convenient management, and high removal efficiency and high purification capacity for pollutants [4]. Therefore, constructed wetlands were used in the present study to remove chlorpyrifos, which is the most organophosphorus pesticide in the world. The results of this study will provide information on the popularization of constructed wetland systems and may be applied in the field of pesticide treatment.
2. Materials and methods

2.1. Experimental setup
As shown in Fig. 1, the bottom of the wetland substrate was filled with 2 cm of gravel with particle size of 6–12 mm as the support layer. In the experiment, the water level was controlled at 2 cm above the substrate; simultaneously, A. calamus was planted evenly in the wetland system.

![Fig. 1 Schematic diagram of the constructed wetland system](image)

2.2. Experimental design
The experiment consisted of three treatment groups: a planted group; an unplanted, non-sterile group; and an unplanted, sterile group (CW1, CW2, and CW3, respectively). The experimental period was set to 7 d, and the experiment was repeated five times for each group and set of conditions. In the experiment, the initial concentration of chlorpyrifos increased from 0.48 mg/L to (0.96 and 1.92) mg/L, in each treatment group.

During the experiment, water and soil samples were collected at the end of each test cycle (i.e., each 7 d), to monitor the chlorpyrifos content in the water and at the soil surface. Soil samples used for detection of the microbial community structure, and the plant samples, were collected at the end of the five repeated experiments. All chlorpyrifos were determined by UV spectrophotometry after pretreatment of water samples, soil samples and plant samples [5-6]. Analysis of microbial community structure using high-throughput sequencing.

3. Results and discussion

3.1. Removal effect of chlorpyrifos in water
As shown in Fig. 2, with an increase in chlorpyrifos concentration, the efficiency of chlorpyrifos removal in water in the CW1 and CW2 system first increased and then decreased. The chlorpyrifos concentration was too low, and no obvious stimulatory effect on plant roots and microbes was observed. This resulted in a lower concentration of chlorpyrifos-degrading enzymes being secreted by plants and microbes [7-8]; thus, the removal efficiency decreased. With increasing concentrations, the level of chlorpyrifos-degrading enzymes secreted by plants and microbes increased gradually [9-10], and the removal efficiency also increased. However, when the chlorpyrifos concentration was too high, the microbial activity was weakened, reducing the absorption of pollutants by plants, and resulting in a decrease in removal efficiency. In the CW3 system, the efficiency of chlorpyrifos removal increased with increasing concentrations. In the unplanted sterile group in the CW3 system, the removal of chlorpyrifos mainly relied on the adsorption of soil substrate, within a certain adsorption capacity, and the efficiency of chlorpyrifos removal increases with the increase in influent concentration.
3.2. Adsorption of chlorpyrifos by soil substrate
As shown in Fig. 3, an increase in the chlorpyrifos concentration resulted in a decrease and then an increase in the amount of chlorpyrifos adsorbed by the substrate in the CW1 and CW2 systems. In the CW1 and CW2 systems, plants and microbes directly absorbed and degraded chlorpyrifos in water, and partially absorbed and degraded chlorpyrifos adsorbed on the surface of substrate[11-12]. At low concentrations, affected by chlorpyrifos-degrading enzymes a lower concentration of chlorpyrifos was consumed through plants and microbes from substrate surface. However, at high chlorpyrifos, the microbial activity was weakened, thus reducing the amount of chlorpyrifos consumed by plants and microbes from the substrate surface, resulting in an increased amount of adsorption. While the CW3 system was within a certain range of adsorption capacity, the amount of chlorpyrifos adsorption increased with the increase in influent concentration.

3.3. Absorption of chlorpyrifos by plants
In order to understand the absorption ability of chlorpyrifos by plants, analysis the contents of chlorpyrifos in plants. As shown in Fig. 4, the amount of chlorpyrifos adsorbed by A. calamus was 0.5, 0.6, and 0.4 mg/g, respectively, where there was no significant difference. As a result of the short experimental cycle, the chlorpyrifos content in A. calamus were low.
3.4. Degradation of chlorpyrifos by microbes

3.4.1. Degradation characteristic of chlorpyrifos by microbes. In order to understand the characteristics of chlorpyrifos degradation by microbes, the CW2 and CW3 systems were compared and analyzed. As shown in Fig. 5, under all concentrations conditions, the efficiency of chlorpyrifos removal in the CW2 system was higher than that in the CW3 system, which indicated that the microbes assisted in the degradation of chlorpyrifos. With increasing concentrations, the differences were 8.3, 12.0, and 5.1%, respectively. When the chlorpyrifos concentration was low, there has no obvious stimulatory effect on microbes, which led to reduced levels of chlorpyrifos-degrading enzymes. This weakened the effects of microbial degradation, so the differences in the removal efficiency was less. With an increase in chlorpyrifos concentration, the concentration of chlorpyrifos-degrading enzymes increased gradually, and the effects of microbial degradation were enhanced, so the differences in the efficiency of removal followed an increasing trend. However, with a further increase in chlorpyrifos concentration, the microbial activities decreased, the chlorpyrifos-degradation effects by microbes were further weakened, and the differences in the removal efficiency showed a decreasing trend.
3.4.2. Richness and diversity of the microbial communities. The results of the statistical sample sequencing process of the high-throughput sequencing information are shown in Table 1, the microbial community diversity was in the order C4 > C1 > C2 > C3. And the microbial community richness was followed the order C2 > C1 > C3 > C4. That is, when the chlorpyrifos concentration was 0.48 mg/L (C2), the microbial community richness in late operation was higher than at the start of operation (C1) and at other concentrations. When the chlorpyrifos concentration increased to 0.96 mg/L (C3) and 1.92 mg/L (C4), the microbial community richness in late operation was lower than in initial operation. Moreover, increase in the concentration of chlorpyrifos caused some microbial communities to be gradually eliminated, and their richness to be decreased.

| Sample | Number of sequences | OTUs | Diversity | Coverage | Richness |
|--------|---------------------|------|-----------|----------|----------|
|        |                     |      | Shannon   | Simpson  |          | ACE      | Chao1    |
| C1     | 54250               | 5684 | 6.72      | 0.013    | 0.96     | 7907.30  | 7462.04  |
| C2     | 57546               | 5866 | 6.70      | 0.015    | 0.96     | 8116.36  | 7684.91  |
| C3     | 53272               | 5242 | 6.64      | 0.013    | 0.96     | 7583.50  | 7287.68  |
| C4     | 51936               | 5421 | 6.90      | 0.011    | 0.96     | 7295.42  | 6983.22  |

3.4.3. Microbial community structure analysis. As shown in Fig.7, to determine in more detail the diversity of the microbial communities in these experiments, only the top 13 microbial communities of each sample were analyzed at the phylum (Fig.7a), class (Fig.7b), and genus (Fig.7c) level. The predominant bacterial phyla in the four samples were Proteobacteria, Acidobacteria, Actinobacteria, Gemmatimonadetes, Firmicutes, Bacteroidetes, and Verrucomicrobia. The relative abundance of the top seven predominant bacterial phyla in sample C1, C2, C3, and C4 was (92.6, 92.4, 93.9, and 92.3) %, respectively. Among them, the relative abundance of Proteobacteria was the highest in each sample. Comparing the initial operation (C1) and later operation (C2, C3, and C4), the abundance of Proteobacteria decreased with the duration of operation. With increase in the initial concentration of chlorpyrifos, the abundance of Proteobacteria increased by late operation, but the difference was not significant.

At the class level, the predominant bacterial classes in the four samples were Alphaproteobacteria, Betaproteobacteria, Actinobacteria, Gemmatimonadetes, and Gammaproteobacteria. The relative abundance of the five predominant bacterial classes in the C1, C2, C3, and C4 samples were (65.4, 48.7, 62.3, and 61.4) %, respectively. The relative abundance of Alphaproteobacteria was the highest in all samples.

At the genus level, the predominant bacterial genera in all four samples were Sphingomonas, Gemmatimonas, Gp6, Subdivision3_genera_incertae_sedis, and Gaiella. The relative abundance of the five predominant bacterial genera in samples C1, C2, C3, and C4 were (25, 22.6, 28.2, and 30.4) %, respectively. Among these five, the relative abundance of Sphingomonas was the highest in all samples. Clostridium sensu stricto was detected in samples from later operation (C2 and C3), and its relative abundance was relatively high (12.21 and 1.36 %, respectively). However, Clostridium sensu stricto was seldom detected in the initial operation (C1) (0.09%) or in C4 (higher initial concentration of chlorpyrifos) (0.15%).
Fig. 6 Microbial community structure and relative abundance of each sample at the level of phylum (a), class (b), and genus (c)

4. Conclusion
In this study, the characteristics of chlorpyrifos removal by the constructed wetland system were studied. Additionally, the microbial community structure in wetland systems was analyzed by high-throughput sequencing technology, as follows: In the wetland system, the removal of chlorpyrifos was accomplished by the combined action of substrate adsorption, plants absorption, and microbial degradation; however, with different initial of chlorpyrifos concentrations and different systems, there were significant differences in chlorpyrifos removal by substrate, plants, and microbes. Through high-throughput sequencing, the microbial community richness in the late stage of wetland system was negatively correlated with the initial concentration of chlorpyrifos. Proteobacteria and Acidobacteria were the dominant phylum in the system, which was beneficial to the removal of chlorpyrifos.

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