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The Effect of LDPE Microplastics on Soil Metabolic Activities and Microbial Community Profile

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Abstract

Almost half a million tons of plastic wastes are produced every year and it is accumulating in the environment. However, there is a lack of understanding of their potential influence on soil metabolic activities and microbial diversity. Therefore, a soil microcosm experiment was conducted to determine the influence of microplastics (MPs) (size ~ 500 μm) on soil microbial activities with various concentrations of low-density polyethylene MPs (0, 0.5, 1, and 2% w/w). PCR-DGGE was applied to determine the influence of MPs on soil microbial community structure. This study revealed that MPs with concentration > 1% could affect soil microbial metabolic activities and bacterial community profile with incubation time. Soil catalase (CAT), dehydrogenase (DHA), and invertase (INV) activities were determined. Soil DHA and INV activities decreased over time. No clear trend or pattern was observed in DHA activities. Catalase activity increased with the concentration of microplastics over time. Furthermore, DGGE analysis showed that LDPE microplastics >1% pose a powerful selection pressure on soil microbial community structure. In particular, microbial communities associated with hydrocarbon degradation were enriched (*Pseudomonas, Bacillus,* and *Sphinogomonas* species) in soil contaminated with a high concentration of LDPE microplastics. These findings add to the growing body of evidence on the complex interference of MPs on the soil ecosystem. The influence of MPs in microbial diversity and activities is a significant environmental concern as it may also disturb N and P nutrient cycling, which needs further investigation.

Keywords: Microplastics; LDPE; Soil enzymes; Soil microbial profile; DGGE

1. Introduction

Plastics have infiltrated nearly all aspects of people's lives. Approximately 380 million metric tons of plastics are produced globally each year, where the majority of these plastics are newly synthesized and they will end up in landfills or the environment (Geyer *et al.,* 2017; Wang *et al.,* 2019). Microplastics (MPs) are defined as plastic particles with a diameter less than 5 mm which originate from the fragmentation of large plastics and direct environmental emission (i.e beads). Recently, many researchers have drawn their attention to microplastics (MPs), especially in the marine environment. Growing bodies of evidence showed direct and indirect detrimental effects of MPs pollution on marine and freshwater biota. Therefore, microplastics are amongst the contaminants of emerging concern which pose a long-lasting threat to global diversity and habitats.

On contrary, little attention has been made to the terrestrial environment although plastics are produced, used, and disposed on land. It has been estimated that soil may have 4 to 23 times more MPs than in the ocean (Wang *et al.,* 2019). MPs enter the soil in various ways such as application of wastewater, landfill leachate, the disintegration of plastic mulch, and atmospheric deposition (Nizzetto *et al.,* 2016).

Concentrations as high as 7% of microplastic (w/w) have been reported in highly contaminated topsoil (Fuller and Gautam, 2016). While studies indicating the ubiquitous presence of microplastics in soil environments are accumulating, their potential effect on soil ecosystems is still limited in knowledge. MPs will persist in soils for hundreds of years due to their stability and inert properties (Shah *et al.,* 2008), which may critically affect soil physicochemical properties and the microbiome. Studies of de Souza Machado *et al.* (2018) showed that MP-induced physical changes in soil affected soil microbial activity. Fei *et al.* (2020) also showed that the addition of polyethylene (PE) and polyvinyl chloride (PVC) inhibited fluorescein diacetate hydrolase activity but stimulated urease and acid phosphatase activities in acid soil.

Moreover, MPs may disturb the soil microbial communities through alteration of soil physical and chemical properties. Studies reported that the microorganisms rapidly form biofilm on MP surfaces with distinctive microbial communities from the surrounding environment (Liu *et al.,* 2018; Huang *et al.,* 2019). The modification of microbial communities in the environment is a significant environmental concern as it may disturb the nutrient cycling and microbial metabolic activities. Thus, we attempt to study the influence of MPs on soil metabolic activities and microbial diversity using low-density polyethylene (LDPE) microplastics. The objective of this study was to investigate the influence of MPs on microbial metabolic activities and soil microbial community structure. The outcome of this study could provide better understanding on the potential influence of MPs in the soil ecosystem.

2. Materials and Methods

2.1 Soil microcosm experiment

An agricultural soil with no history of plastic pollution was purchased from Ratchaburi province, Thailand. The soil was sieved at 2 mm to remove all rocks and plant debris. The physical and chemical characteristics of the soil were analyzed by the Department of Soil Science, Faculty of Agriculture, Kasetsart University, Thailand. In brief, the soil texture was determined by hydrometer method (Allen *et al.,* 1974), the soil pH by pH meter, soil organic matter by wet oxidation method (Walkley and Black, 1934), total nitrogen by Kjeldahl method, available phosphorus by Bray II method (Bray and Kurtz, 1945), available potassium by flame photometry. The soil was a neutral clay soil (pH 7.2) with 2.32% organic matter, 980 mg kg^{-1} total nitrogen, 29 mg kg^{-1} available phosphorus, and 239 mg kg^{-1} available potassium. A soil microcosm experiment was conducted in a closed system using a 125 ml glass vial containing 50 g of agricultural soil added with 0, 0.5, 1, and 2% low-density polyethylene microplastics (500 µm, Alfa Aesar, Thermo Fisher Scientific). It has been reported that polyethylene is the most common form of plastics that are used in the agricultural area including greenhouse cover, irrigation tubes, mulching films, and packaging (Horton *et al.,* 2017). The initial water holding capacity was adjusted to 50% for each microcosm for standard heterotrophic respiration assessment (Comeau *et al.,* 2018). Triplicate samples were then incubated at 30°C in the dark for 60 days. The soil samples were collected periodically to determine the microbial activity.

2.2 Soil enzyme assay

The catalase (CAT) activity was measured by titration method using 0.2 M $KMnO_4$ (Stpniewska *et al.,* 2009). The activity was expressed in mL 0.2 M $KMnO_4$ g^{-1} soil 20 min^{-1}. The dehydrogenase (DHA) activity was determined with 2(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) following the method of Yang *et al.* (2021). The results were expressed in terms of µg INTF g^{-1} soil 24 h^{-1}. Soil invertase (INV) activities were measured by using dinitrosalicylic acid (DNS) methods Ma *et al.* (2014). All measurements were carried out in triplicate.

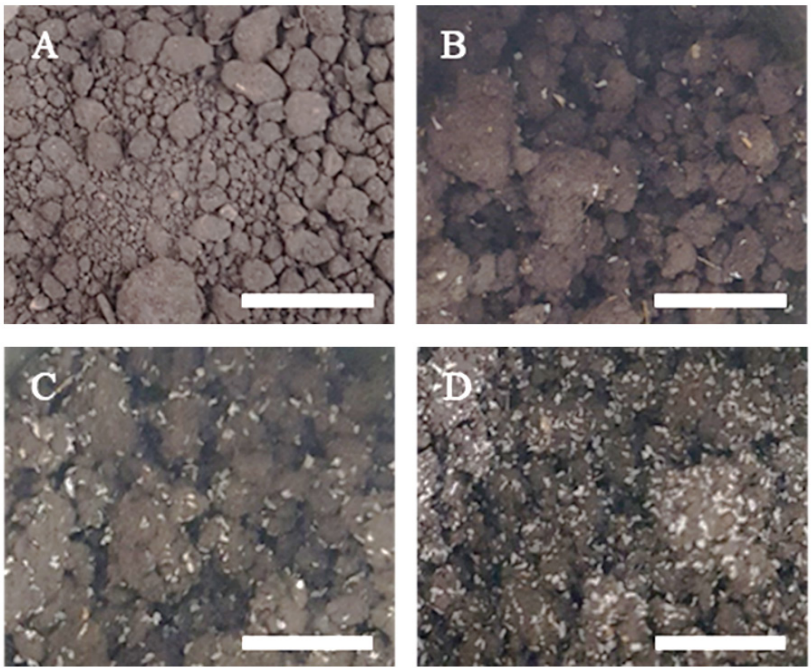

Figure 1. The physical structure of soil integrated with microplastics (white dots in B, C, and D). Where, (A) is control soil (B), soil with 0.5% MPs, (C) soil with 1% MPs, (D) soil with 2% MPs. The white bar represents 1 cm in size.

2.3 Soil microbial fingerprinting

PCR-DGGE was applied to determine MP-induced changes in soil microbial diversity. The soil genomic DNA was extracted using DNeasy® PowerSoil® Kit (Qiagen, USA) following the manufacturer's directions. The V3 region of 16S rRNA gene fragments was amplified using 341F (GC-clamped) and 518R primers. The denaturant gradient gel electrophoresis (DGGE) analysis was carried out with the DGGE-2001 system (CBS Scientific Company, USA). The PCR- amplified V3 region of gDNA was loaded onto 8% acrylamide gel with a denaturant gradient from 45% to 65%. The DGGE was performed at 80V, 60℃ for 14 h. Photographs were obtained from Gel Doc (E-Box VX2/20M, Vilber Lourmat, Marne-la-Vallée, France). Predominant bands were excised, re-amplified, purified, and then sequenced through Mahidol University-Osaka University Collaborative Research Center for Bioscience and Biotechnology (MU-OU: CRC, Bangkok).

2.4 Statistical analysis

Data were analyzed by one-way ANOVA using SPSS 18.0 statistical software package. Pearson's product-moment correlation was used to determine the association between the parameters. The analysis was done at a 95% confidence level ($p < 0.05$) by the least significant difference (LSD). All values from the analysis were presented as the mean ± standard deviation.

3. Results and Discussion

3.1 Effect of MPs on bacterial community structure

The activities of soil CAT, DHA, and INV in soil amended with various concentrations of MPs are shown in Figure 2 The results showed that the presence of MPs significantly affected soil CAT, DHA, and INV activities. A significant increase ($p < 0.05$) in catalase activities was observed compared to the control treatment (without MP) shortly after

10 days of incubation. Furthermore, we have observed a significant increase in catalase activity with an increasing concentration of MPs. Catalase is an enzyme that indicates the activity of aerobic microorganisms (Stpniewska *et al.,* 2009). Aerobic respiration is generally higher soil with high porosity, which leads to enhanced gaseous diffusion and water holding capacity. It has been reported that the respiration rate in clay loam soil is approximately 50% higher than in sandy soil (Kowalenko *et al.,* 1978). Recent studies demonstrated that MPs alter soil physical properties which consequently affect soil metabolic activities (de Souza Machado *et al.,* 2018; Huang *et al.,* 2019). Huang *et al.* (2019) reported that an increase in catalase and urease activity when they amended microplastics to the soil, which could be attributed to an increase in soil porosity and aggregate structure of the soil, similar to our results.

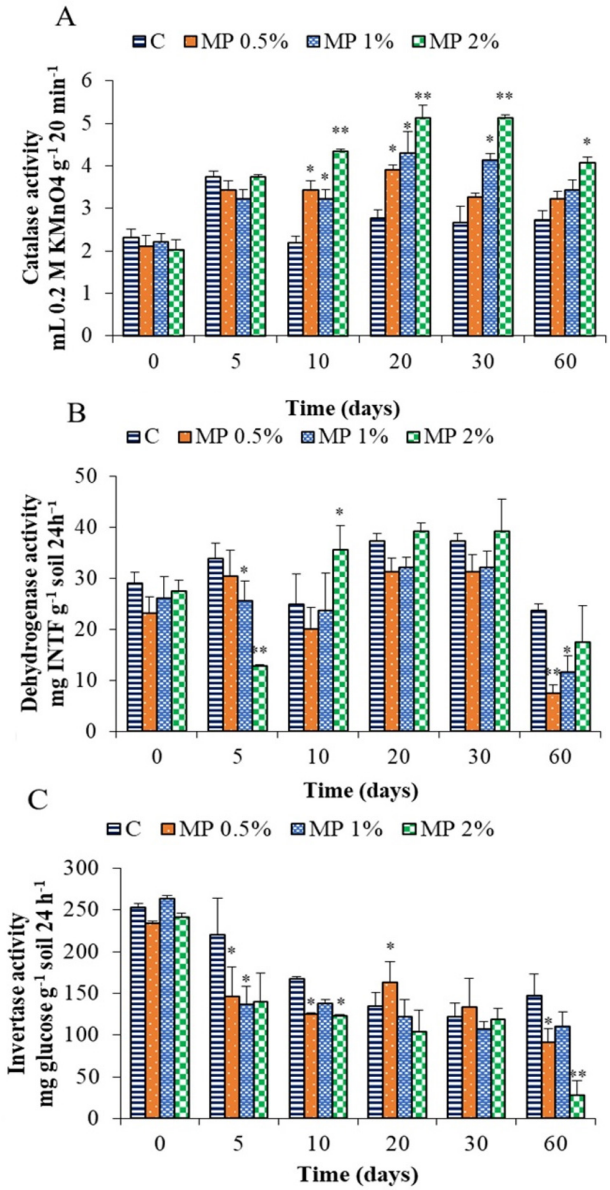

Figure 2. Soil enzyme activities during the period of incubation, (A) Catalase activity, (B) Dehydrogenase activity, (C) Invertase activity

The soil dehydrogenase represents the soil respiration and health of the soil (Kaczyńska *et al.,* 2015). We have observed that DHA activity fluctuated throughout the incubation period regardless of MP concentration. The alteration in soil respiration is closely related to all biogeochemical processes, which may lead to an imbalance in nutrient cycling disturbing the whole soil ecosystem (Rillig *et al.,* 2021). Although the precise mechanism on how MPs disturb soil metabolic process is still limited, it is clear that the presence of MPs alters soil physical properties and therefore altering soil metabolic process.

On the other hand, a significant decrease ($p < 0.05$) in soil INV activity was observed which was concentration-dependent. Invertase is a critical enzyme involved in the decomposition of organic compounds in the soil, which reflects the carbon cycling process of soil (Ma *et al.,* 2014). Currently, it is believed that the addition of microplastics in the soil alters the C: N ratio which is known to affect the decomposition rate (Melillo *et al.,*1982; Rillig *et al.,* 2021). This may reduce the transformation of soil organic matters to simple carbons which serve as a viable carbon source for soil microorganisms.

3.2 Effect of MPs on bacterial community structure

The DGGE profile from MP amended treatments showed distinctive banding patterns and intensities from the control treatment at 1 and 2% MP after 60 days of exposures (Figure 3). In contrast, no visible difference was observed for 0.5% MP compared to the control treatments. The dominant species in 1% MP were *Sphinogomonas* sp., *Bacillus* sp. and uncultured bacterium, and *Phreatobacter* sp. *Moorella humiferrea, Pseudomonas* sp., and *Bacillus* sp. in 2% MP amended treatments. *Pseudomonas, Bacillus* and *Sphinogomonas* species often dominate in hydrocarbon-contaminated soil (Bacosa and Inoue, 2015). As LDPE are hydrocarbon polymers, it is possible that these species colonized and dominated to utilize LDPE as a carbon substrate. The enrichment of *Pseudomonas, Bacillus,* and *Sphinogomonas* species under MP contamination implies that these bacteria may play a role in biofilm formation and degradation of LDPE MPs. Similar results were observed by Delacuvellerie *et al.* (2019) where they saw enrichment in microbial communities associated with hydrocarbon degradation when LDPE microplastics were amended. Huang *et al.* (2019) also reported that the relative abundance of amino acid metabolism and xenobiotic degradation was higher in MP amended soil compared to the normal soil. Our study indicated that microplastics provide a distinctive habitat to indigenous microorganisms which alters microbial community structure and subsequent soil metabolic process. However, it is unclear to what extent this alteration will affect soil nutrient cycling and decomposition of organic matter in the soil.

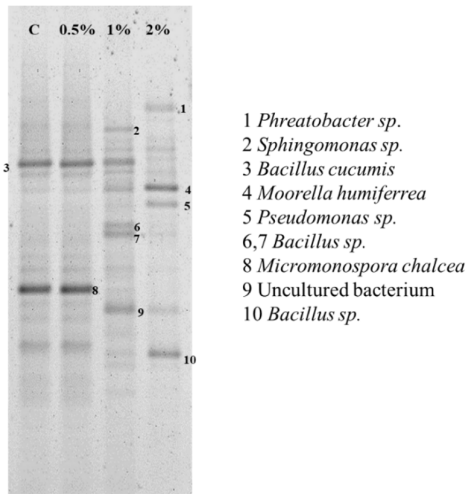

Figure 3. Microbial profile after 60 days of exposure of MPs

4. Conclusion

A microcosm experiment was performed to determine the effect of LDPE microplastics on microbial metabolic activities and bacterial diversity. The results demonstrated that microplastics affect the soil microbial enzyme activities indicating a disturbance in the C cycle. Furthermore, DGGE analysis showed that MPs > 1% significantly altered the microbial community structures by allowing communities associated with hydrocarbon degradation to dominate such as *Pseudomonas, Bacillus,* and *Sphinogomonas* species, which presumably are microbial communities that may degrade LDPE microplastics. Further investigation on how different shapes, types, concentrations of microplastics impact different soil ecosystems is urgently needed to understand the potential threat of MPs on the soil ecosystem. These results contribute to a better understanding of the effects of LDPE microplastics on soil microbial communities and their respective metabolic activities.

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