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Microplastic Can Decrease Enzyme Activities and Microbes in Soil

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Abstract

An *in vitro* study was conducted to investigate the impacts of microplastics on enzyme activities and soil bacteria. The study included four different treatments of microplastics including a control. Different levels of microplastics were applied to the soil ranging from 0% to 5%, to assess the impacts of microplastics on soil enzymes and subsequent soil bacteria. After 30 days of incubation, the soil samples were collected and growth parameters of bacteria were assessed. Activities of *β*-glucosidase, urease and dehydrogenase enzymes were also determined. Our results showed that the presence of microplastics in the soil significantly reduced bacterial population together with bacterial strains. The activities of *β*-glucosidase, urease and dehydrogenase enzymes were reduced significantly to approximately 32%, 40% and 50% in microplastics treated soils respectively. Concentration of microplastic has a role to play towards this direction; the higher the concentration of microplastic the greater is the impact on enzymes and soil bacteria. The present study on the microbial soil health *vis-à-vis* microplastic application indicates that the material can have negative effect on the soil bacterial population of and thus ultimately may jeopardize soil health and crop production.

Keywords

Microplastic, Concentration, Enzyme Activity, Bacteria, Crop Production

1. Introduction

As a group of anthropogenic contaminants, microplastics are globally recognized as pervasive and persistent. Microplastic is widely studied in marine ecosystems [1] [2] [3], and only in recent years has attention shifted to terrestrial ecosystems [2] [3]. Microplastics are tiny particles of plastics with size smaller than 5 mm [4], with various shapes (e.g., fiber, fragment, film) and polymer types (e.g., po-

lyester, polyethylene, polyacrylic, polypropylene), which are intentionally produced or fragmented into micro-sized plastics by natural and/or anthropogenic factors, microbial degradation or plowing [5].

Polyethylene (PE) is a polymer widely used to produce mulch films and other plastic products used in agriculture [6]. Low density polyethylene (LDPE), a synthetic resin manufactured by polymerizing ethylene, is used in agriculture, such as in greenhouses and for mulching. LDPE is commonly used because of its versatility, processability, low cost, and flexibility [7] [8]. All these advantages make it an ideal raw material to achieve benefits such as maintaining soil temperature and moisture content and preventing weed growth, all of which ultimately contribute to enhanced agricultural production [9]. However, the widespread use of non-biodegradable LDPE has resulted in serious environmental concerns. Recent research has shown that the presence of LDPE microplastics in the soil can alter soil physico-chemical characteristics [10] [11] [12] [13]. Moreover, LDPE has been recognized as a substrate for distinct microbial colonization, which may modify the microbial community structure and hinder ecosystem functioning [14]. These changes are likely to alter the fertility of the soil.

The quantity of microplastics in the soil is an important factor in determining the physico-chemical and microbiological properties. Studies by Machado *et al.* [11] and Zhang *et al.* [12] found no remarkable shifts in the soil microbial community with 1% (w:w) polyetylene, polyvinyl chloride, or polyethylene terephthalate microplastics compared to the controls. In contrast, 5% (w:w) polyvinyl chloride microplastics considerably increased the abundance of microbes [15]. However, these findings vary among different studies. Few studies [16] [17] observed that the polystyrene microplastics decreased microbial population. Correlations among LDPE microplastics concentrations, soil biological properties and microbial communities have rarely been analyzed. Thus, knowledge of the relationships between these and their underlying mechanisms remains incomplete, impeding our capacity to address the issues associated with microplastic pollution in agroecosystems.

We hypothesized that LDPE microplastics may alter soil microbiological properties that these changes could differ with varying concentrations of LDPE microplastics. Thus, the aim of this study was to explore the effect of LDPE microplastics across a range of concentration levels on changes in 1) Soil bacterial richness, diversity, and abundance; 2) Soil enzyme activities; 3) Correlations among bacterial communities, enzyme activities and LDPE microplastic concentrations. The results of this study enhance our understanding of the potential risks posed by LDPE microplastics in agroecosystems. In addition, our findings will be useful for policymakers to develop policies and regulations to minimize plastic-associated environmental issues and to protect soil health.

2. Material and Methods

2.1. Study Area

Soil sample was collected from the top 20 cm from an arable field located at

Mymensingh (Upazila: Bhaluka), Bangladesh. It is a terrace soil belonging to the Noadda-1 soil series. According to the USDA [18] soil taxonomy the soil is Ultic Ustochrept and the land type is high land. The bulk of soil samples were collected by composite soil sampling method and processed. To date most of the studies regarding microplastic were focused on the soil contaminated with industrial wastes. Arable soil was often neglected. Thus, we selected an arable field to observe the impacts of microplastics on arable soil.

2.2. Soil Sample Preparation

The soils were air-dried, visible roots and plant debris were discarded and the soils were ground gently to break up larger soil aggregates. After that the soils were sieved at 2 mm, thoroughly homogenized and finally characterized. Physico-chemical properties of the soil (pH, texture and organic matter content) were determined with the standard method described by Rowell [19]. The soil had a loam texture with a pH of 5.24 and organic matter content of 1.63%. The moisture content of the soil was 13.2%.

2.3. Generation of Microplastics

Microplastics were generated using a cryogenic grinder and liquid nitrogen [20] [21]. Low density polyethylene (LDPE) sheets were purchased from Hebei Qiudu Technology Co., Ltd. located in Hebei, China. These plastic sheets were cut into pieces (1 × 1 cm) using scissors. A 100 ml grinding vial was filled half with the plastic pieces, followed by placing the vial in the cryogenic grinder. The lid of the cryogenic grinder was closed. After that, the vial was submerged in liquid nitrogen for 15 minutes for precooling. The plastic was then agitated using the grinder. The grinding procedure consisted of eight cycles of 2 min grinding and 2 min cooling, with a grinding rate of 12 cps (cycles per second). Finally, the vial was opened using the vial extractor and microplastics were collected for further experimental use.

2.4. Incubation Study

An incubation experiment was conducted following a randomized block design to investigate the impacts of LDPE microplastic on soil microbiological properties. The incubation experiment was based on four groups of microplastic treatments *viz*., control, 0.05%, 0.50% and 5.00% (w/w) which were selected on the basis of values of microplastics found in arable soil [6]. All treatments were replicated four times (n = 4). Each container contained 400 g air-dried soil. LDPE microplastic was added to the soil according to the treatments. After adding LDPE microplastics to the soil, the soils were thoroughly mixed and homogenized with a glass rod to distribute the microplastics as evenly as possible. 100 ml of deionised water was added to each container and thoroughly mixed into the soil to establish a soil water content of 25% w/w. The containers were wrapped within plastic film to prevent evaporation. Finally, all containers were kept at

30˚C for an incubation period of 30 days. Deionised water was added (0.5 - 1.0 g) on a weekly basis to maintain a constant water content. After 30 days the soil was collected to determine bacterial isolates, bacterial population, β-glucosidase, urease and dehydrogenase enzymes activities.

2.5. Laboratory Analysis

Various physical, chemical and physico-chemical properties of the soil sample were analyzed [19]. Total viable count (TVC) of bacteria was enumerated manually by the number of colonies forming units. TVC was determined following the pour-plate technique (Obenauf and Finazzo). Following streaking and incubating, single bacterial colony was isolated. Inocula from the colonies were sub cultured in slants and bacteria were tested for purity for morphological parameters; and when homogeneity of a single isolate was ensured, a number of morphological and biochemical tests were done for identifying the bacteria. Morphological tests involve the observation of bacterial colony (color, shape, size, elevation and transparency). Biochemical tests include catalase, oxidase, nitrate reduction, TSIA (Triple Sugar Iron Agar), LIA (Lysine Iron Agar) and KIA (Kligler Iron Agar). Finally bacterial isolates were identified using "Bergey's Manual of Determinative Bacteriology" [22].

The β-glucosidase activity was estimated by using p-nitrophenyl-β-D-glucoside (PNG) as a substrate and incubating 1 g of soil with 0.25 ml toluene, 4 ml modified universal buffer (pH 6), and 1 ml PNG solution (25 mM) for one hour at 37˚C. After incubation at 37˚C, 1 ml of $CaCl_2$ solution and 4 ml Tris buffer (pH 12) were added, and absorbance was taken at 400 nm using a spectrophotometer [23]. The urease activity was determined by using urea as a substrate. Five grams of moist soil was incubated with 1 ml methylbenzene, 10 ml of 10% urea 20 ml citrate buffer (pH 6.7) for 24 hours at 37˚C. One milliliter of filtered soil solution, 1 ml of sodium phenolate, and 3 ml of sodium hypochlorite were added and diluted to 50 ml, and absorbance was determined at 578 nm using a spectrophotometer [11] [24]. Dehydrogenase activity was measured using triphenyl tetrazolium chloride (TTC) as a substrate where the TTC solution (0.3 - 0.4 g/100 ml) was mixed with 5 g of moist soil and incubated for 24 h at 30˚C. The triphenyl formazan (TPF) formed was extracted with acetone and measured spectrophotometrically at 546 nm. Finally the dehydrogenase activity was expressed as μg TPF g^{-1} dry soil h^{-1} [23] [24].

2.6. Quality Control and Statistical Analysis

All data were analyzed using SigmaPlot (version 14) software. Data were tested for normal distribution using the Shapiro-Wilk and Kolmogorov-Smirnov test and equal variance using Levene's mean test [25] [26]. Data were normally distributed for all analyses except urease activity. Thus, within the urease activity dataset, data were square root transformed. A one-way analysis of variance (ANOVA; treatment) was used to detect the differences in bacterial population,

β-glucosidase, urease and dehydrogenase enzymes activities across four microplastic treatments. Pearson correlations were performed to determine how bacterial population, β-glucosidase, urease and dehydrogenase enzymes activities related to each other.

3. Results

3.1. Identification of Bacteria

After the end of incubation period, the bacteria present in the control and microplastic treatments were identified. Total 22 bacterial strains were identified in the soil control treatments (Table 1). However, bacterial strains were found to be reduced by microplastic treatments. Three bacterial strains were (*Bacillus alvei*, *Bacillus thuringiensis*, *Bacillus krulwichiae*) were disappeared in 0.05% treatments. Likewise eight (*Bacillus subtilis*, *Bacillus bataviensis*, *Bacillus alvei*, *Bacillus thuringiensis*, *Bacillus krulwichiae*, *Azotobacter macrocytogenes*, *Azospirillum lipoferum*, and *Azotobacter armeniacus*) and 16 (*Bacillus subtilis*, *Bacillus*

Table 1. Bacterial isolates present in control treatments.

Number of strain	Bacteria	Color	Shape	Size (mm)	Elevation	Transparency
1	*Bacillus subtilis*	yellow	cocci	0.2	convex	opaque
2	*Bacillus bataviensis*	white	cocci	0.1	raised	opaque
3	*Bacillus aneurinilyticus*	gray	spiral	0.5	convex	opaque
4	*Bacillus alvei*	white	irregular	0.2	umbonate	opaque
5	*Azotobacter macrocytogenes*	white	irregular	0.5	raised	opaque
6	*Bacillus thuringiensis*	yellow	rod	0.5	umbonate	opaque
7	*Azospirillum lipoferum*	blue	spiral	0.2	convex	opaque
8	*Bacillus krulwichiae*	white	rod	0.2	convex	translucent
9	*Azotobacter armeniacus*	yellow	irregular	0.3	raised	opaque
10	*Azotobacter indicum*	yellow	rod	0.2	flat	opaque
11	*Azospirillum lipoferum*	green	rod	0.5	umbonate	opaque
12	*Micrococcus luteus*	white	cocci	1.0	convex	translucent
13	*Bacillus siralis*	white	cocci	0.5	umbonate	opaque
14	*Bacillus sphaericus*	yellow	cocci	1.0	umbonate	opaque
15	*Azotobacter chroococcum*	blue	rod	0.3	flat	opaque
16	*Azotobacter agilis*	blue	spiral	0.2	raised	translucent
17	*Xanthomonas campestris*	green	irregular	0.5	flat	opaque
18	*Paenibacillus apiarius*	white	spiral	1.0	convex	opaque
19	*Bradyrhizobium japonicum*	blue	irregular	0.3	convex	opaque
20	*Azospirillum amazonense*	yellow	cocci	2.0	convex	opaque
21	*Azotobacter bryophylli*	blue	cocci	0.2	flat	opaque
22	*Bradyrhizobium elkanii*	white	spiral	2.0	convex	translucent

bataviensis, *Bacillus thuringiensis*, *Bacillus krulwichiae*, *Bacillus sphaericus*, *Azotobacter macrocytogenes*, *Azotobacter indicum*, *Azotobacter chroococcum*, *Azotobacter bryophylli*, *Micrococcus luteus*, *Xanthomonas campestris*, *Paenibacillus apiaries*, *Bradyrhizobium japonicum*, *Bradyrhizobium elkanii*, *Azospirillum lipoferum*, and *Azospirillum amazonense*) bacterial strains were disappeared in the 0.50% and 5.00% microplastic treatments respectively.

3.2. Total Viable Count (TVC) of Bacteria

Bacterial colonies started to appear after 48 hours of incubation in soil and microplastic treated inocula indicating the presence of bacteria in these materials. Control soils had a total viable count (TVC) of 90×10^6 CFU/ gm. Microplastic treatments significantly affected the TVC; TVC was significantly ($p \leq 0.05$) reduced by 4%, 13% and 21% in 0.05%, 0.50% and 5.00% LDPE treatments respectively (Figure 1).

3.3. Enzyme Activities

All three enzyme activities (β-glucosidase, urease and dehydrogenase) in the present experiment closely followed the pattern of the total viable count in various treatments. The AVOVA test indicates that the treatment had highly significant ($p \leq 0.05$) effect on enzyme activities. The control soil had higher activities of β-glucosidase, urease and dehydrogenase compared to the treatments. β-glucosidase activity was significantly ($p \leq 0.05$) reduced to 10%, 31% and 54% in 0.05%, 0.50% and 5.00% LDPE treatments respectively (Figure 2(a)). Urease activity was significantly ($p \leq 0.05$) decreased to 6%, 14% and 30% in 0.05%, 0.50% and 5.00% LDPE treatments respectively (Figure 2(b)). Likewise dehydrogenase activity was significantly ($p \leq 0.05$) reduced to 24%, 41% and 60% in 0.05%, 0.50% and 5.00% LDPE treatments respectively (Figure 2(c)). Strong positive correlations ($p = 0.00$) were observed between bacterial population and β-glucosidase, and between urease and bacterial population (Table 2). Negative correlations were observed between β-glucosidase and urease, between β-glucosidase and

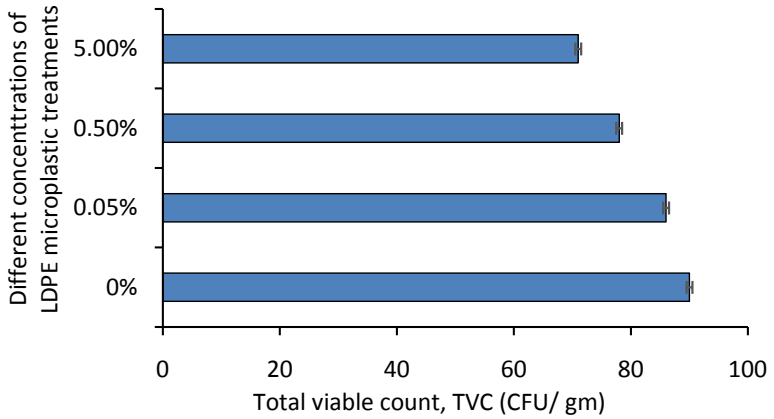

Figure 1. Total viable count (TVC) of bacteria in soils treated with different doses of LDPE microplastics.

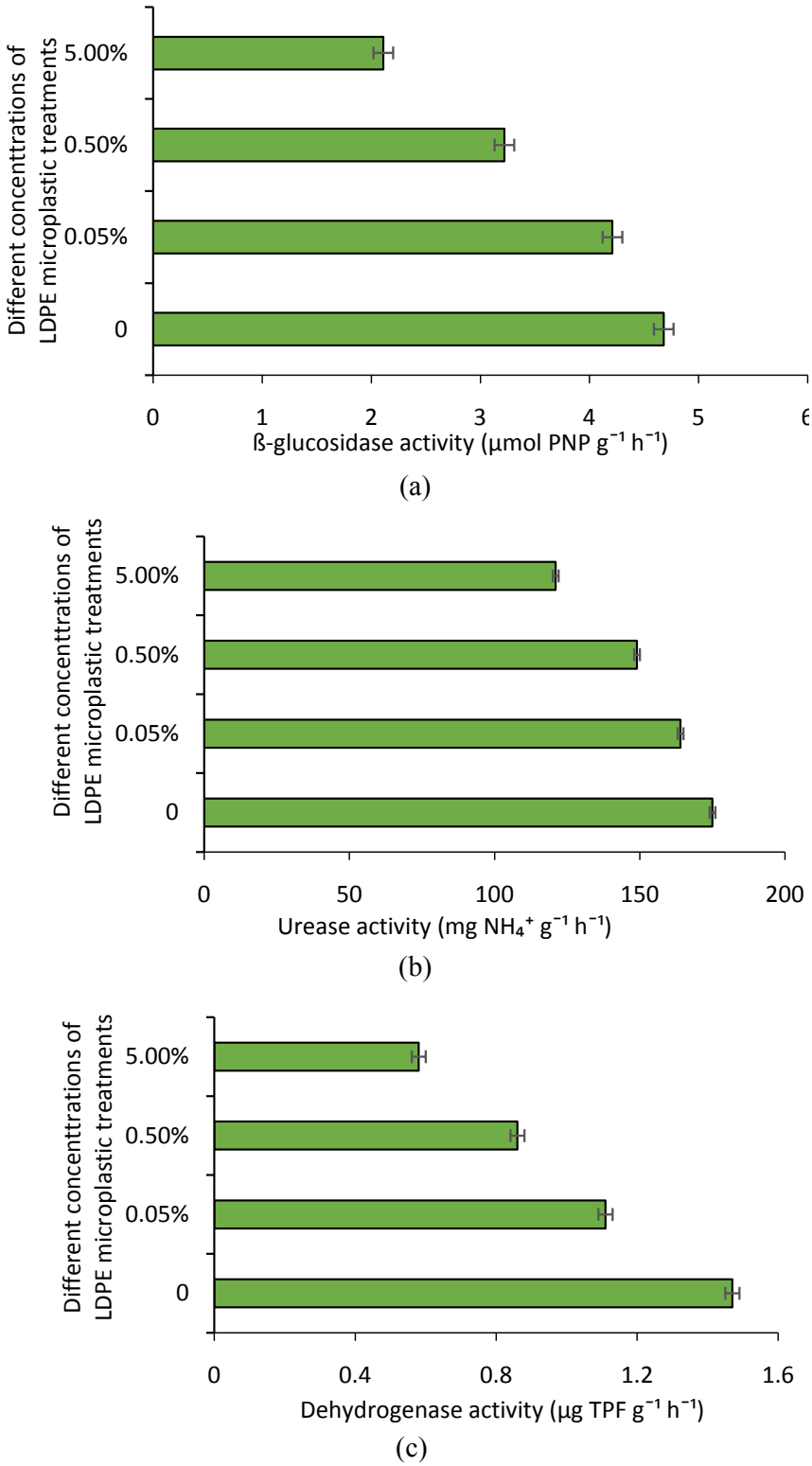

Figure 2. (a) β-glucosidase, (b) urease and (c) dehydrogenase enzyme activities in soils treated with different doses of LDPE microplastics.

dehydrogenase. However, no relationship was found between urease and dehydrogenase (Table 2).

Table 2. Correlations of bacterial population, β-glucosidase, urease and dehydrogenase enzyme activities. Upper values indicate Pearson correlation coefficients; lower values indicate p values. Values in italic are significant at $p \leq 0.05$.

Bacterial population	β-glucosidase		
	0.921		
	0		
		Bacterial population	
Urease	−0.832	0.974	
	0.0003	0	
			Urease
Dehydrogenase	−0.723	0.981	−0.517
	0.0004	0	0.079

4. Discussion

Number of bacterial strains and total viable count (TVC) count of bacteria were reduced in LDPE treated soils compared to the controls which might be due to the antagonistic effects resulting from microplastics. The antagonistic effects could result from increased adsorption of nutrients and decreased activity of soil enzymes. Studies showed that microplastics in the soil can decrease enzymatic activity [12] [27] [28] which was in agreement with our study. Authors showed a declining trend in enzyme activities with the increasing level of microplastics and explained that the microplastics block enzymes and substrates required in breakdown of complex compounds which ultimately leads to decreased sorption of nutrients [27] [29]. Another probable explanation for the reduced enzyme activity could be the incorporation of microplastics into the dynamic structure of aggregates introducing fracture points into the aggregates, and thus decreasing aggregate stability. Water extractable carbon (WEC) is used as an index of micro and macroaggregates [30]. Decreased aggregate stability could adversely impact on WEC which would impact on soil bacterial population.

Microplastics tend to reduce the soil microbial respiration [31]; reduced microbial respiration is linked with reduced enzyme activity which could result in lower bacterial population [32]. Reduced number of bacterial strains and TVC in our study supporting the hypothesis of [33] explaining that increased soil alkalinity can significantly reduce microbial diversity and richness. However, authors conducted the experiment in marine environments instead of soil. Our findings were consistent with previous literatures [11] [12]. Soil pH does not influence respiration directly, rather it controls nutrient solubility and availability which impacts on soil bacterial population responsible for soil organic matter decomposition which is evident by soil respiration [33].

Study [13] [34] observed that the microplastics reduce available phosphorus concentration in soil. Reduction in available phosphorus can be linked to the high soil pH. As pH increases above 6 in soils most of the dissolved P reacts with

Ca forming calcium phosphates [35]. These reactions convert the dissolved phosphorus species into insoluble compounds (precipitates). Thus the phosphorus becomes unavailable for the bacteria. Phosphorus is required by the bacteria to some extent for the storage and transfer of biological information, energy metabolism, and membrane integrity [36]. Microplastic has a tendency to decrease aggregate stability [13] and poor aggregate stability would impact on soil enzyme activities and bacterial population. Aggregate serves as a habitat for soil microbes; good soil structure promotes better air circulation and water flow in the soil [37]. Previous studies showed a declining trend in aggregate stability with the increasing level of microplastics. However, it was apparent from the studies [11] [12] [38] [39] that microplastic type and shape are not always likely to be the dominant factor for influencing aggregate stability, and that the characteristics and concentration of the additives present in microplastic are more likely to be the factors affecting aggregates. Effect of microplastics on aggregate stability may decrease water flows [40] in the soil that may explain the decreases in enzymatic activities as observed in the present study.

5. Conclusion

This study examined how the microplastics impact on soil microbiological properties. The experiment was designed with four levels of microplastic treatments which are considered environmentally relevant for soils exposed to industrialization. This study shows that LDPE microplastics exerted negative effects on soil microbiological properties. The microplastics had the potentiality to significantly decrease microbial population, bacterial strain, β-glucosidase, urease and dehydrogenase enzyme activities. The higher the concentration of microplastic in soil, the greater the impacts are on soil properties. Given the negative impacts of microplastics in soils, it therefore seems likely to reduce plant growth and development. A more detailed investigation into the soil microbiological properties would be required coupled with plant parameters to cast further light on this. As the impacts of microplastics depend on the concentration levels of LDPE, it seems likely that the impacts could vary with microplastic type and incubation time. Further researches are required to find out how microplastics impact soil microbiological properties in presence of different types of microplastics and incubation periods.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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