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To whom it may concern:

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Promotional Effects and Risks of Multi-Walled Carbon Nanotubes on Phytoremediation of Plastic Films

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Abstract **-** Alfalfa is a representative plant for remediating pollutants in soil, but its rate of plastic degradation rate remains low. Porous materials are often utilized as additives to improve pollutant degradation rates, yet the use of emerging nanomaterials (NMs) has not been tested. This study aims to combine alfalfa and carbon nanomaterials (CNMs) to research the degradation effect on double-layer polyethylene (PE) plastic films (PF) in soil. Meta-analysis was used to select multi-walled carbon nanotubes (MWCNTs) with low phytotoxicity from various NMs. In a 90-day dynamic experiment, the combination of 100 and 200 mg/kg MWCNTs and alfalfa significantly improved the PF degradation rate in both surface and bottom soil layers. In particular, the PF degradation rate of the surface layer, with the direct participation of alfalfa roots, reached a degradation of 11.08±1.34 %. This suggests that a degradation system incorporating alfalfa and MWCNTs holds promising potential for plastic phytoremediation.

Keywords**:** Plastic; Phytoremediation; Biodegradation; Nanomaterials; Meta-analysis

1. Introduction

Plastic, despite being one of the most used materials globally, is struggling with issues of neglect and environmental contamination [1]. Plastics directly inhibit agricultural production, deterioration of the soil environment, and reduction of fertility [2]. The rhizosphere system of plants serves as a critical channel not only for the transport of nutrients [3], but also for pollutants like plastics to enter plants [4]. Increasingly, research has focused on the toxicity of plastics on plants and rhizosphere systems including microorganisms.

At the same time, the degradation of organic pollutants represented by plastics has become a topic that has been studied. Some degradation pathways, such as volatilization and redox, can be accelerated by human intervention. However, these treatments have the disadvantages of high energy consumption, cost and secondary pollution [5]. Compared with these treatment methods, biodegradation can not only achieve the adsorption of pollutants and destroy their structure, but also has the advantages of being environmentally friendly and cost-effective [6]. Plants, such as legumes are often used in bioremediation [7]. Legumes, represented by *Medicago sativa* (alfalfa) and *Glycine max* (soybean), have complex root systems and nodules, which provide a favorable rhizosphere environment for the growth of potentially degrading microorganisms to achieve effective phytoremediation, especially rhizoremediation [8].

Recently, nanomaterials (NMs) have found broad applications in optics and electronics [9, 10], while their application in pollution degradation fields, especially photodegradation, has been gradually increasing in recent years [11, 12]. Meanwhile, several studies have attempted to enhance plant and microbial growth performance by introducing additives against the stress caused by pollutants, including NMs [13], compost [14], biochar [15], and nitrogen [6]. However, the potential threats to plants from NMs with high concentrations and large particle sizes have also been investigated [16, 17].

Therefore, meta-analysis was used to identify a type of NM with the lowest potential toxicity to plants. A 90-day dynamic experiment was conducted to determine whether NMs can promote the degradation of polyethylene (PE) plastic films (PF) in soil by alfalfa.

2. Materials and Methods

2.1 Materials

The materials for the phytoremediation experiment, including PF, pots, nutrient soil, gravel, and alfalfa seeds, were purchased from Amazon UK. Black double-layer PE film was selected as the contaminant. Multi-walled carbon nanotubes

(MWCNTs or CNTs) with diameters ranging from 5 - 20nm and consisting of 4 to 8 layers were purchased from Shilpent.

2.2 Selection of NMs

Meta-analysis was used to summarize and analyze the data provided by the literature on the reported phytotoxicity of NMs. The methodological steps of meta-analysis have been elaborated in our previous study [18].

2.3 Phytoremediation experiment

Phytoremediation experiments were conducted using potted plants in a greenhouse at University College London. The total duration of the experiment was 90d (days). 200mg/kg of PF was evenly distributed at two depths within the flowerpot: 0-2cm from the top (referred to as Surface PF) and 0-2cm from the bottom (referred to as Bottom PF). Soil mixed with either 100 or 200 mg/kg of MWCNTs was added into the flowerpots as two experimental groups, called CNTs100 and CNTs200. Alfalfa seeds were sterilized using 70% ethanol, and dewhitened, then evenly sown on the surface of the soil. A schematic diagram of a whole pot is shown in Figure 2. The three control variable groups included variations where no MWCNTs, no alfalfa, or neither MWCNTs nor PF were added. Each group had three parallel samples. At timepoints of 30^{th}, 60^{th} and 90^{th} d, the pots were destructively sampled to determine plant growth indicators and residual PF. The residual amount of PF was quantified using the weighing method, with a recovery rate of 100%. Data visualization was performed using R and Origin software.

3. Results and Discussion

3.1 Selection of NMs

Analyzing data of 702 samples from 27 studies on the interaction between plants and NMs, Figure 1 shows the effects of various NMs on different plant growth indicators, The results indicate that plant growth indicators in the control group are significantly higher than those in the NMs group ($p<0.01$). Similarly, the Metal NMs (MNMs) group also shows a significant difference ($p=0.01$) compared to the control group, with 100% of the MNMs groups' center points (i.e., mean of the group) situated on the left. This suggests that MNMs generally inhibit plant growth, even at low concentrations such as 0.006 mg Ag/L[19]. In contrast, Carbon NMs (CNMs) tend to show more promotory[20, 21]. In Figure 1, the red diamond representing the CNMs group is located on the right side of the vertical axis, indicating that the growth indicators of the control group are significantly lower than those of the NMs group ($p<0.01$). Moreover, 40% of CNMs group center points are on the left side, while the remainder are on the right side with a promoting effect on plants, especially MWCNTs. Therefore, MWCNTs, as a type of NMs with generally less toxic effects on plants[22, 23], were chosen as additives in pot experiments. Further discussion on the phytotoxicity of NMs can be found in our previous[18].

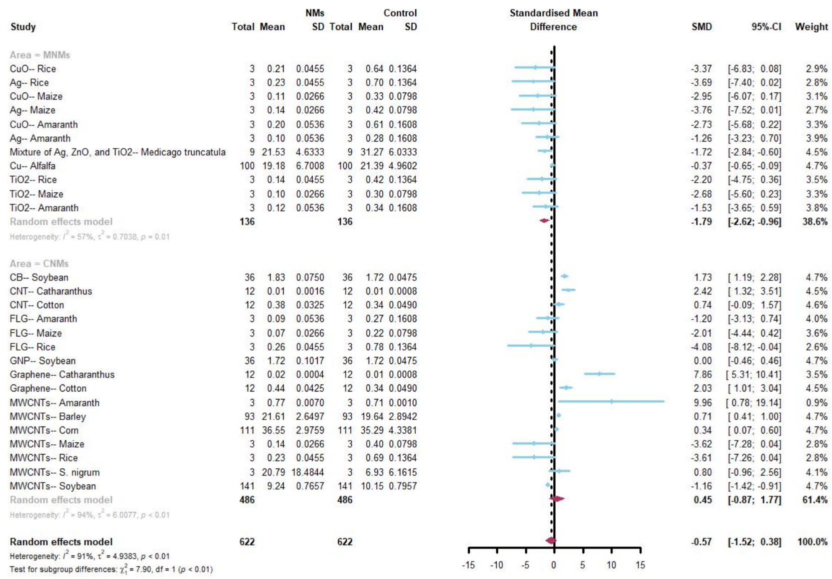

Figure 1 Quantitative analysis of different nanomaterials' toxicity effects on plants growth. The blue horizontal line represents the confidence interval of the NMs and corresponds to the 95%-Cl on the right. Horizontal lines completely on the left or completely on the right side of the vertical line (invalid line) represent that the experimental group is significantly smaller or larger than the control group, otherwise there is no significance. The red diamond represents the overall data of the group, and the significance judgment is the same as the horizontal line. The dotted line represents the position of the mean of the whole data, so that it is easy to compare with each group of data.

3.2 Growth data

Within the range of 0-90d, the moisture content of each group remained consistent, and the pH was maintained at 7.5±0.5 without manual adjustment. Figure 2(a) shows the structure of a flowerpot used in the potting experiment, while Figure 2(b) shows the growth indicators of alfalfa at the 90th d. The biomass of alfalfa significantly increased when 200 mg/kg of MWCNTs was added, compared to when 100 mg/kg of MWCNTs or no MWCNTs were added. Additionally, the root length and plant height of this group were higher than those of the other two groups, although there was no significant difference between the two experimental groups with added MWCNTs. Moreover, the number of flying insects captured on the potted insect-catching plates followed the same trend as the biomass at the 90th d. Before this time point, differences in all these parameters were not significant. This suggests that 200mg/kg of MWCNTs can promote plant growth and reduce the number of flying insects in the soil over the long term like 90 d, consistent with the results of the meta-analysis as shown in Figure 1. There are several potential reasons for the capacity of CNTs to enhance plant growth. On one hand, CNMs can promote the uptake of nutrients by plants from the soil [17]. On the other hand, CNMs can reduce stress from pollutants [24], as well as reducing the effects of plant diseases and pests [22]. This aligns with the observation that CNTs can accelerate the degradation rate of PF as shown in Figure 3 and decrease the population of flying insects in the soil.

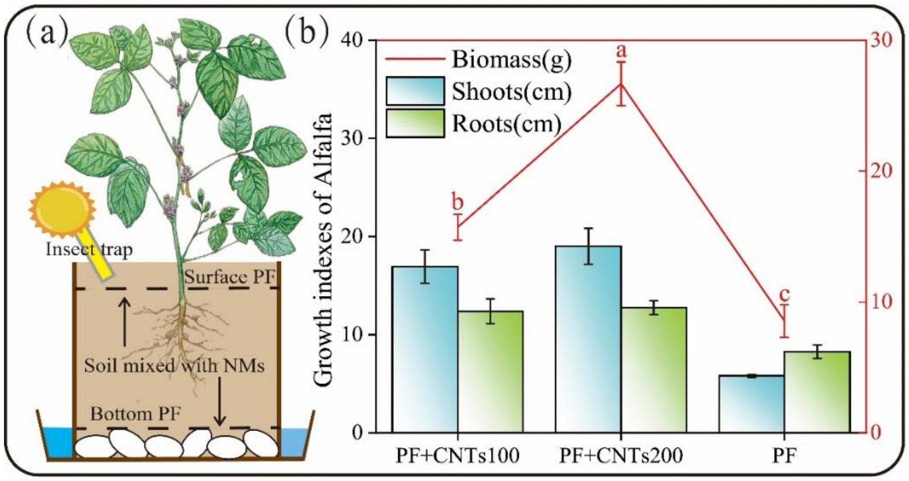

Figure 2 (a) The schematic diagram of a pot; (b) Growth indexes of alfalfa at the 90th d.

3.3 Degradation data

In the range of 0-90d, the maximum degradation rate of the groups with only added PF and PF+alfalfa did not exceed 1.88±1.33%. Therefore, Figure 3 directly shows the dynamic change curves of the degradation rates of Bottom PF and Surface PF in CNTs100 and CNTs200 groups. By the 90th d, the combined effect of 200 mg/kg CNTs and alfalfa resulted in the Surface PF degradation rate reaching 11.08±1.34%. This degradation rate is higher than other Surface PF plant degradation efficiencies reported before[25]. During 0-30d, 200 mg/kg CNTs improved the degradation rate of PF compared to 100 mg/kg CNTs, both at the bottom and on the surface. During 30-60d, both 100 and 200 mg/kg CNTs significantly increased the degradation rate of Surface PF compared to Bottom PF. This indicates that CNTs and alfalfa may be the main degradation promoting factors during the 0-30d and 30-90d periods, respectively. Possible reasons for this include increased rhizosphere activity (including roots and microorganisms) [26], pollutant bioavailability [27], and plant physiological functions [28] within the “alfalfa-CNTs” system. Therefore, CNTs and alfalfa may promote the degradation of PF together through one or more pathways.

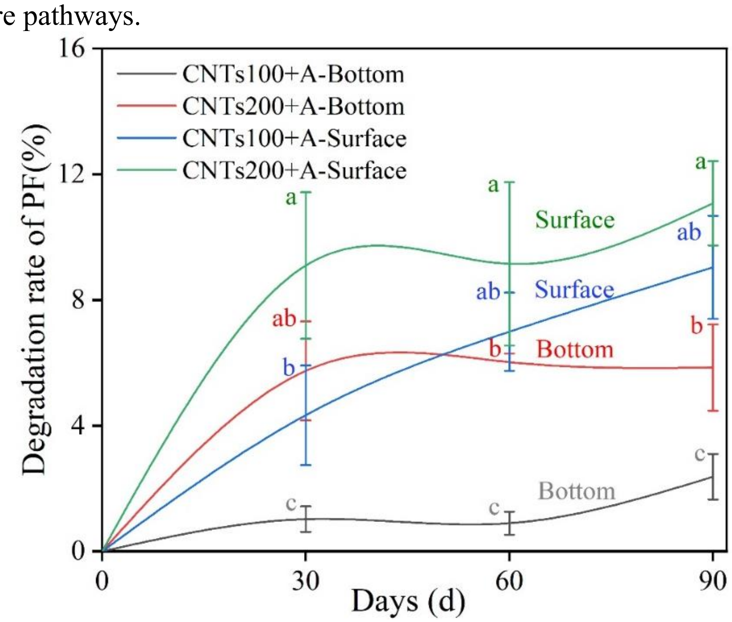

Figure 3 Dynamic degradation curves of Surface and Bottom PF over 0-90d.

4. Conclusion

As a new type of material, NMs have the potential for application in phytoremediation. Meta-analysis indicates that MWCNTs exhibit lower phytotoxicity than MNMs and other CNMs. Therefore, MWCNTs were introduced into alfalfa pots containing PF. A 90-day dynamic test showed that CNTs can significantly improve both growth indicators of alfalfa and the degradation rate of PF, particularly for PF on the soil surface accessible to plant roots. This shows that the combination of alfalfa and CNTs has a high potential for phytoremediation of plastics.

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