SYNTHESIS AND CHARACTERIZATION OF MICROPLASTIC ACCUMULATE FUNGAL PATHOGEN IN ECOSYSTEM

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ABSTRACT

Various microorganisms, including pathogenic ones, have been shown to colonise MP, a common contaminant in nature. Fungi have been generally ignored in this setting, despite their affinity for plastics and their significance as pathogens. Mycobiomes from municipal plastic waste from Kenya were analysed using ITS metabarcoding and a comprehensive meta-analysis, and visualised using scanning electron microscopy and confocal laser scanning microscopy to understand the role of MP as a carrier of fungal pathogens in terrestrial ecosystems and the immediate human environment. The plastisphere core mycobiome was generated by a range of fungal species, including major animal and plant diseases, found in the plastisphere metagenome and microscopic findings. These artificial microhabitats, known as MPs, are capable of attracting a variety of fungal communities, as well as certain opportunistic human infections, such as Phoma-like Cryptococcus. The presence of fungal infections in soil conditions necessitates that MP be considered a persistent reservoir and possible vector. In light of the increasing volume of plastic garbage in terrestrial ecosystems around the world, this connection may have serious ramifications for fungal diseases' global epidemiology.

1. INTRODUCTION

To begin with, plastic garbage has become an unavoidable feature of the Anthropocene; it is now a major source of pollution in the natural world.This means that plastics can harm both aquatic and terrestrial species. In addition to entanglement and suffocation, larger plastic debris can cause intestinal obstruction in organisms. Invertebrate and vertebrate species take up microplastic particles (MP), the most common type of piece, leading to widespread bioaccumulation. To complicate chemical communication in aquatic systems and bioamplify potentially harmful xenobiotics in the food chain, plastic solid waste (PSW) may produce an eco-corona by adsorbing several hydrophobic organic compounds. Because MP may be consumed, breathed in, or touched on the skin, humans are continually exposed to it. Respiratory infammation and lung disease, as well as endocrinological diseases, are all possible side effects of inhaled particles because of their intrinsic toxicity as well as chemical leachability. Despite the fact that plastic pollution is a hot topic, little is known about the interactions between microorganisms and plastics. The so-called "plastisphere," a protective biological niche formed by plastic waste's hydrophobic surface, is an ideal environment for microbial colonisation and biofilm production. On plastics from marine and limnic environments as well as from fluvial ecosystems in different biomes from the Equator to the Polar Regions, these epiplastic communities have been shown to contain archaea and bacteria as well as unicellular and oligocellular eukaryotes, including mushrooms. Metabosomal analyses have shown that MP favours distinct microbial communities, whose succession is affected by factors such as geographic location, time of year, and the polymer type. MP is a microhabitat with high selectivity and plasticity that can affect ecosystems, such as the spread of antibiotic resistance or changes in microbial nitrogen and carbon cycle dynamics through community structure shifts.

Fungi are an appropriate group of organisms for examining microbial plastic colonisation in terrestrial environments because of their apical growth, invasive growth forms, biofilm production, and secretion of hydrophobic proteins (hydrophobins). There is no evidence to suggest that soil-deposited plastic debris cannot be colonised by most higher phylogenetically (non-zoosporic) fungi because they do not depend on the aqueous phase for their growth and produce significantly

more biomass than prokaryotes. Drifting MP20, which is a part of polymicrobial biofilms, is colonised by fungi early on, and they have also been found on landfill plastics. Microorganisms such as Rhodotorula and Candida have been found on the plastic surfaces of medical and industrial equipment as well as domestic gadgets. Fungal pathogens may be accumulated and propagated in soil habitats, such as home gardens, roadsides, agricultural soils, and landflls, that receive a massive infusion of PSW, such as Tus, MP. So far, investigations into the effects of MP on aquatic systems and distant locations have not taken into account the immediate human context.

One of the most important aspects of microplastics' impact on the environment is the study of microbial life on their surfaces. For example, microplastics serve as habitats for bacteria, including pathogenic ones, locations for biofilm formation, and transportation mechanisms for microbes. It is possible for microorganisms that grow on microplastics to harm the environment and human health. Surface-attached microorganisms (SAPs) can interact, proliferate, change characteristics, and biodegrade in the presence of microplastic particles (MPPs). Even though plastic pollution and microplastic pollution have been widely discussed, the impact of plastic on microbial life has yet to be fully analysed.

1.1.1 In marine environments, fungi are part of biofilms associated with plastic debris.

Marine fungi are generally understudied, which is also reflected in the relatively small number of studies targeting marine fungi on marine plastic debris (MPD). In the North Sea, seasonal comparisons of microbial communities, including bacteria and fungi, were conducted on submerged PET bottles, glass slides, and seawater. Ascomycota, Basidiomycota, and Chytridiomycota were the major fungi in the community. Researchers found seasonal and geographic differences in PET-attached eukaryotic groups, including fungus. For this study, we exposed PE and PS to Baltic Sea waters and the River Warnow to see if fungi could colonise the surfaces of these materials. Numerous Chytridium species, the Rhinosporideacae fungus, Rhizidiomyces taxa, and Pythium species were detected on both types of plastic. Unclassified fungi were ascribed to a significant percentage of the sequences.

Although polymer type had no effect on the fungus community composition, location had a considerable impact. In addition, the alpha diversity of PE and PS was much smaller than that of the nearby water and wood particles. A 44-week trial at a North Sea harbour and an offshore site used PE plastic sheets and dolly ropes to weigh down close to the sand. In line with Baltic Sea research, they discovered that Ascomycota were the most common, followed by Basidiomycota and Mucoromycota. Additional members of the Lecanoromycetes class (Physconia, Candelariella, and Caloplaca) of the Ascomycota were identified. Researchers found considerable differences in the beta diversity of the fungal community composition between natural substrates and plastic polymers, as well as between the environments in which they were exposed. Cladosporium cladosporioides and Fusarium redolens, two previously recognised PE degraders in terrestrial contexts, were found.

1.1.2 Fungi's Plastic Biodegradation Capabilities

Degradation by living organisms is known as biodegradation, particularly by microbes. The bacteria ingest or assimilate the biodegradation's soluble products (usually low molecular weight substances). Any amount of biodegradation is better than none at all. It is also called biomineralization when the process of biodegradation has resulted in the production of carbon dioxide. However, in the absence of a terminal electron acceptor, organic matter decomposition results in the creation of CH4 and/or other short-chain hydrocarbons, conditions that are countered in some reduced environments. It is microorganisms that release enzymes or other chemicals (such as acids and peroxides) that facilitate biodegradation in natural environments. Prolonged exposure to these synthetic substances can cause fungi to breakdown them. This includes persistent organic pollutants and polycyclic aromatic hydrocarbons (PAH) as well as pesticides such as neonicotinoid pesticides (NCPs) or organophosphorus pesticides (OPPs). Biodegradation of plastics in the environment could be a metabolic feature of some fungi because of their metabolic diversity and capacity to digest complicated chemicals. Aspergillus, Fusarium, and Penicillium are all members of the Ascomycete phylum, which includes some known plastic-degrading fungi. Hydrolyzing PET to TPA (terephthalic acid) was accomplished by F. Solani, a member of the collection.

1.1.3 Microplastics pose a danger to both animals and humans.

The role of microplastics in marine life and their impact on marine dwellers has been extensively investigated in recent years, but only a limited amount of research has been done to investigate the impact of microplastics on human health. People are thought to consume up to millions of MP particles each year in the form of several milligrammes of microplastic per day. Humans are exposed to this contamination through the consumption of freshwater natural resources that contain microplastic particles (MPs). This is due to the fact that water treatment using coagulation-flocculation techniques can only remove up to 88 percent of MPs.Microplastic particles ranging in size from 440 to 275 parts per million (ppm) have been found in Chinese tap water. The amount of microplastics in water from natural sources ranged from 1473 to 3605 particles per litre, while the amount of microplastics in treated drinking water ranged from 338 to 628 particles per litre. The microplastics made up of PET, PP, and PE accounted for 70% of the total number of microplastics. Humans can be exposed to microplastics and nanoplastics in a variety of ways, including ingestion, inhalation, and direct skin contact. According to studies, the average person consumes between 39,000 and 52,000 microplastic particles per year through food, with seafood carrying microplastic being one of the primary sources.

2. REVIEW OF LITERATURE

Katja Wiedner (2020). There are numerous sources of microplastic and microglass particles in aquatic and terrestrial settings. For example, the influence on soil microorganisms is unknown, and the environmental impact is difficult to measure. This is why we conducted an incubation experiment with 1% of five distinct types of contaminants (less than 100 micrometres) in an agriculturally utilised soil (Chernozem). Polypropylene (PP), low-density polyethylene (LDPE), polystyrene (PS), polyamide 12 (PA12) and microglass were the impurities. We used phospholipid fatty acids (PLFAs) as markers for bacteria, fungi, and protozoa after 80 days of incubation at 20 C to study the soil microbial community structure. According to the data, soil microorganisms were not adversely affected by the presence of microplastics and microglass. While untreated soil has a lower PLFA concentration than treated soil, PLFA concentrations in treated soil increased with the addition of

LDPE (28%), PP (19%), and microglass (11%). PLFAs in treated soil dropped with the addition of PA12 (32%), and PS (11%). PLFAs showed significant differences in PA12 (89 percent) and PS (43 percent) compared to LDPE in terms of PLFA concentrations. After 80 days of incubation, bacterial PLFA variability was substantially larger than that of fungi, despite the presence of various contaminants in the environment. For protozoa, the results showed that treatment with microplastic had little effect, as evidenced by the small decrease in PLFA content when compared to controls in this group. Microglass, on the other hand, appears to inhibit protozoa, as PLFAs were below the limit of determination when it was used. Our investigation found that high concentrations of different microplastics could have distinct effects on soil microbiology. Protozoa may be poisoned by microglass.

Hurley and colleagues (2018): A wide variety of consumer and industrial items, such as abrasives, fillers, film, and binding agents, can be made using microplastics. A wide range of sources and paths for microplastics into the environment makes identifying and quantifying their sources and pathways extremely challenging and time-consuming. While synthetic polymer identification and quantification in sediments and water have been developed, analytical methodologies for soil matrices are either unavailable or in an experimental stage.

Machado et al. (2018) The bulk density, water-holding capacity, hydraulic conductivity, soil aggregation, water-stable aggregates, and microbiological activity of soil were all affected by a 2-percent concentration of microplastics in the soil. When microplastic particles are present in soil environments, a wide range of reactions can be triggered. This extensive investigation explains these processes. The subject of microplastics does not currently include microglass, despite the fact that glass is extremely resistant to corrosion or weathering. Blasting abrasives, fillers, and road marking additives all make use of microglass. The way microplastics and abrasives from highways enter the environment is comparable to that of this substance. Both the consequences for terrestrial ecosystems and the effects of microplastics are still unknown.

Olena Stabnikova (2020) Polyethylene, polypropylene, and other common microplastics polluting our waterways have lower densities than water. The neustonic

layer near the water-air contact contains them together with dissolved or colloidal organic debris, hydrophobic cells, and bacterial spores; this is because they are all in the neustonic layer. Biofilms of hydrophobic and often potentially harmful bacteria could coat the floating micro- and nanoparticles of plastic.This could generate environmental and public health problems. Aquatic animals find biofilm-coated microplastics more appetising than plain microplastics, which amplifies microplastics' harmful effects. The accumulation of microplastics in the water-air interphase and their interaction with bacterioneuston necessitates consideration of the environmental implications of even modest amounts of microplastics. In order to interact, grow, change characteristics, and biodegrade, microorganisms attached to the surface of microplastic particles may employ the particles as substrates for their own growth and replication. The study of microbial life on the surface of microplastic particles is one of the most important aspects of their environmental role.

Gerhard Rambold (2021) biotrophic-antagonistic on plants, animals, or both, with the ability to live a pathogenic life in any PCM species.In the PCM (except A. crassa), all species have strains that have been reported as opportunistic human pathogens that can infect different parts of the human body, including the skin and/or other superficial areas (superfial, cutaneous), subcutaneous tissue (subcutaneous), and deep tissue and multiple organs (invasive, systemic). Remotididymella anthropophila, a Phoma-like fungus recently discovered from a clinical sample of the human respiratory tract, was the most prevalent fungal species on MP, accounting for more than 13% of reads, followed by the phytopathogenic Leptosphaerulina australis and the multi-host infecting Phoma herbarum.

3. MATERIALS AND TECHNIQUES.

3.1 A sample collection of plastic waste

On Lake Zurich's beach at Wa denswil (UTM coordinates 32T 474250 5231960), trash was found in September 2015. In the reed belt, the plastic bits were detected at a depth of up to 15 centimetres. A 0.7 cm x 0.7 cm piece of plastic was cut off by using tweezers to pick up the shards of plastic. A sterile 50-ml Falcon tube was then used to store the sample. To ensure their safety, tweezers and scissors were sterilised with 75% ethanol and a lighter before each use. The falcon tubes were carried to the lab in

a refrigerated bag and maintained at 5°C until they were needed. A single piece of plastic was found along the lake's edge, out of a total of 13 collected. Only a 2.5 cm2 part of one piece of hard plastic could be removed using tweezers.

3.2 Isolation of fungal spores

A single piece of plastic debris was placed in each Falcon tube, and 3 mL of sterile water was added in the lab. The fungus hyphae and spores were separated from the plastic samples by mixing the tubes in a vortex mixer for around 15 seconds. Using a sterile pipette and a flamed glass rod, we applied 100 l of water from each Falcon tube to a Petri plate with modified Melin-Norkrans (MMN) nutritional agar on the sterile bench. It was necessary to incubate a total of four Petri dishes in a Falcon tube. A few days later, the first fungus colonies could be seen on the plates after being cultured at room temperature and in the dark. Colonies of emerging fungi were then punched out with a flamed hook, transferred to malt agar medium in glass tubes (dubbed "test-tubes"), and maintained at room temperature in complete darkness.

3.3 Identification of fungi

Mycelial development slowed down to a trickle after fungal mycelia covered nearly half of the agar in the glass tubes. The isolated fungal strains were morphologically categorised according to their exterior appearance in terms of colour and texture, with the goal of selecting fungi for DNA identification. A subset of the nuclear small subunit rDNA was sequenced from representative strains from each of these subgroups. Each well in a 96-well plate of PCR-grade water was filled with 100 ul of DNAse/RNAse-free water, and fungal mycelia samples were added to each well. It was then frozen and thawed at ambient temperature three times to liberate the DNA from the fungal hyphae, which was done by soaking them in liquid N2. Once this solution had been diluted 1:10 in PCR-grade water, it was PCR-grade water and the primer pair ITS3/ITS4 were utilised as templates for the PCR reaction. It was next necessary to run the nucleotide sequences against the National Center for

Biotechnology Information's (NCBI's) database to determine which species was most closely related to the sequences generated by the PCR reactions. The fungi that could breakdown PE or PU were sequenced again using the primer pair ITS1/ITS4 in order to obtain longer DNA fragments for a more accurate identification. The NCBI GenBank has these nucleotide sequences.

3.4 Fungus species from the fungal collection for degradation assays

Fungi from various ecological guilds, such as common saprotrophs (e.g. Agaricus bisporus), wood- and tree-rot saprotrophs, tree pathogens (e.g. Heterobasidion parviporum), and ectomycorrhizal fungi (e.g. Suillus granulatus), were tested for their ability to degrade polyethylene (PE), polypropylene (PU), and polyethyleneWhite rot" and "brown rot" fungi were distinguished by their ability to breakdown lignin, while "brown rot" fungi were unable to do so.

4. CONCLUSIONS

As soon as water from the Falcon tubes was dispensed onto the Petri dishes, fungus strains began to flourish. More than 100 fungal strains were identified. The fungal strains were classified into morphological categories based on their appearance. One or two fungal strains were chosen from each of these categories; a small subunit rDNA segment was sequenced from one of these selected strains. Some plant diseases and a commonly occurring saprotrophic fungus were among the species found in plastic waste. Cladosporem and Penicillium grisefulvum are two of the more common saprotrophic fungus species. Phialemoniopsis curvata and Xepiculopsis graminea are two fungi that have been shown to be saprotrophs in soils and sediments. Arthrinium arundinis, Leptosphaeria sp., and Phoma sp. are just a few examples of fungi that have been shown to coexist alongside lakeshore plants and grasses. Endophytic fungus Stagonospora neglecta is highly specialised for the common reed (Phragmites australis).Known as a necrotrophic fungus, Botryotinia fuckelinana affects a wide range of plants. Pseudorobillarda texana, Exophiala bonariae, and Setophoma vernoniae, which had a low level of identification, were discovered by others in rocks or on the leaves of alien plants. The common reed pathogen, oomyceteous Pythium phragmitis, was the sole organism not in the fungi group (Phragmites australis).

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