



Assessment of Microplastic Degrading Potential of Fungal Isolates from an Estuary in Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author JOW supervised the study, performed the statistical analysis and managed the literature searches. Author NTO managed the analyses of the study, wrote the protocol and first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The potential of fungi as bio degraders of micro plastic particles was assessed using standard microbiological and Fourier transformed Infra-Red (FTIR) spectroscopic analysis methods. The highest mean Total Heterotrophic fungal (THF) count of 4.24×10^4 cfu/ml was obtained with the least THF (2.72×10^4 cfu/ml) recorded during the dry season. Mean hydrocarbon Utilizing Fungal (HUF) count was highest (1.78×10^4 cfu/ml) during the wet period while the least HUF count (1.46×10^4 cfu/ml) was recorded during the dry period. Spectra of FTIR showed that the water contained microplastic particles in these proportions; polyethylene of low density (LDPE) 0.01%, 0.11% protein, 0.15% polystyrene, 0.37% polyamide, 1.14% cellulose, 1.21% polyurethane, 1.90% polyvinyl chloride, 3.11% polyester and 92% polypropylene, respectively. Species of fungi identified were *Aspergillus niger*, *Penicillium* spp., *Rhizopus* spp., *Mucor* spp., *Aspergillus nidulans*, *Fusarium* spp., *Microsporum canis*, and *Aspergillus fumigatus*. Among the fungal isolates, *A. niger* and *A. fumigatus* were most active in degrading the micro plastic (polypropylene) with mean % weight loss of 71.09% and 53.09%, respectively while the least active was *Penicillium* spp. with

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a mean % weight loss of 28.64% during the study period. The order of degradation was *Aspergillus niger* > *A. fumigatus* > *A. nidulans* > *Fusarium* spp. > *Rhizopus* spp. > *Microsporum canis* > *Mucor* spp. > *Penicillium* spp. The potential to degrade micro plastic particles by these fungi can be harnessed. The foremost active fungi degrading potentials are as follows: *A. niger* (71.1%) and *A. fumigatus* (53.1%). The study has shown that isolates of fungi from Ohiakwu River in Nigeria possess the ability to degrade micro plastic (polypropylene) particles. Therefore, this research is of tremendous importance for industrial development and additionally for future research purposes.

Keywords: *Micro plastic; bio degrader; fung; FTIR spectroscopy.*

1. INTRODUCTION

Plastics are ubiquitous and among the most common and persistent organic pollutants in modern days [1,2] and are globally distributed across aquatic environments [3], reflecting the success of plastic as both industrial and domestic products and their persistence within the environment [4]. Their visibility in litters of many land and water environments poses an excellent challenge for environmentalists, microbiologists and the global community. Plastic debris is categorized as macro plastics (particle diameter > 25mm), small plastics (< 5mm) or meso-plastics (5 - 25mm) [5,6,7].

Micro plastics are artificial organic solid particles with normal or abnormal shapes and with size ranging from 1µm-5mm [8] getting from the chemical process of monomers extracted from petroleum [9,10,11], that cannot dissolve in water from primary or secondary sources [12].

Primary microplastics come from factory-made beads of microscopic size, e.g., pre-produced powders for textile coatings, resin pellets, toothpaste and blasting, microbeads in cosmetics and drug delivery media [13] whereas, the secondary micro plastics are got from the degradation/weathering of larger (i.e., meso- and macro-) plastics [14] due to photolytic, mechanical fragmentation and biodegradation [15].

Industries such as Nigerian Agip Oil Company (NAOC), Port Harcourt Wastewater treatment plant, several sand dredging companies, abattoir house and others as well as illegal waste dump sites are located around the Ohiakwu River. Hence, most of their waste materials are discharged into the River.

Preventing the discharge of plastic polymers into our environment is quite impossible, it is thus necessary to find ways for microbes to degrade these compounds. Using microbes to degrade plastic particles will increase their rate of

microbial degradation while not inflicting harm to the environment [16,17,18] (Islam et al., 2011). Therefore, identifying and use of microorganisms that can utilize microplastics is a promising and eco-friendly strategy that may assist the natural bioremediation in improving the clean-up of the natural ecosystems without adverse impacts. This study is therefore designed to assess the microp1astics degrading potential of fungal isolates from the Ohiakwu Estuary in Rivers State.

2. MATERIALS AND METHODS

2.1 Study Area and Collection of Water Sample

A total of forty (40) samples of surface water were collected for five months during the wet and dry seasons from Ohiakwu Estuary, which has a common boundary with the Rivers State University, Nigerian Agip Oil Company (NAOC), and Eagle Island in Nkpolu-Oroworukwo, Port Harcourt. It is a brackish water ecosystem influenced by tidal fluxes with mangrove vegetation interspersed in some areas with *Nypa* plants and lies within the rectangular coordinates of N4°46'50", N4°48'10", and E6°57'10", E6°57'30" (Fig. 1). Many human activities going on within and around this river include sand dredging, waste disposal, boating navigation, disposal of excreta, to mention but a few. This aquatic body receives effluent discharges from the Port Harcourt Wastewater treatment plant and abattoir house sited by the riverbank.

2.2 Fungal Analysis

The fungal count was determined with sterilized surface-dried Sabouraud dextrose agar using the spread plate method. Aliquots (0.1 ml) of appropriate decimal dilutions were inoculated onto the solidified Sabouraud dextrose agar in duplicates and incubated at 25°C for 72hours. Mineral salt agar (MSA) was used for

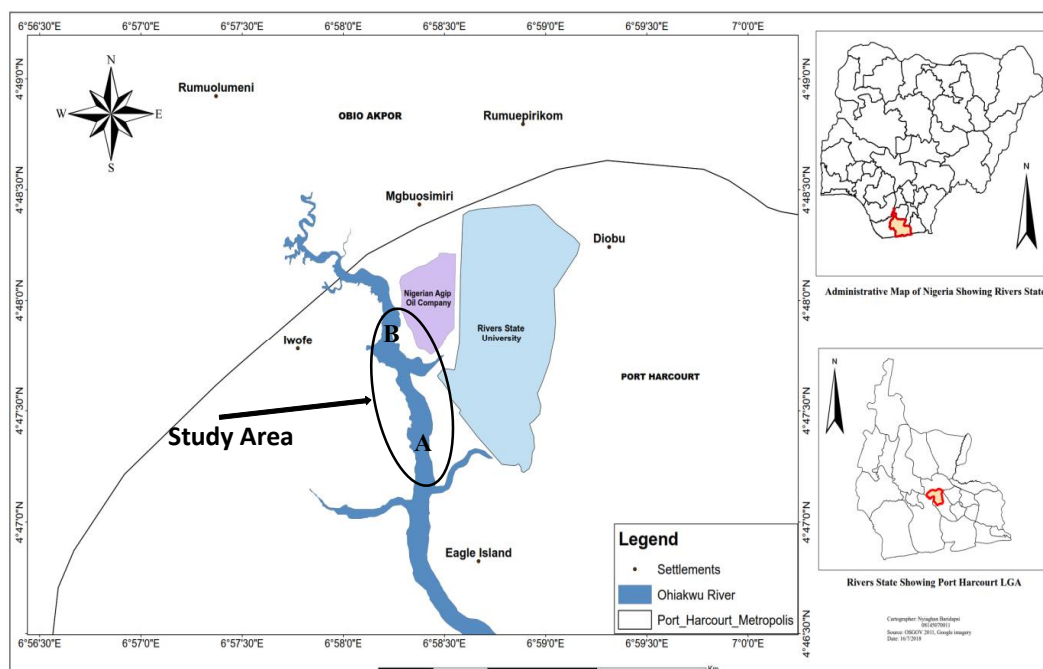


Fig. 1. Map showing study area

hydrocarbon utilizing fungi and incubated at 30°C for 5 days. Discrete colonies were counted as colony-forming units (CFUs) from plates with colonies ranging from 30-300 and representative colonies were sub-cultured onto sterile Sabouraud dextrose agar plates to obtain pure cultures which were preserved in slants overlaid with 10% glycerol for further identification. Test. Isolates of fungi were subjected to identification for their morphological and microscopic features [19,20,21].

2.3 Extraction of Microplastics from Water Samples

Collected water samples from the Estuary were placed in clean beakers of 1-litre capacity. Filtration and separation based on the weight (density) of plastic particles was achieved by saline (NaCl) solution, followed by the methods adopted by [22,23]. Approximately 800 ml of 1.2 g NaCl solution (w/v) was added to the beaker for micro plastics separation via floatation by increasing the density of the solution. The solution was mixed and kept overnight and the overlying water was directly filtered with Whatman Grade 1 filter paper of 11µm pore size. Then filters were placed into clean Petri dishes and covered until further analysis.

2.4 Identification and Confirmation of Micro Plastics

The filters were subjected to virtual inspection and larger micro plastics were sorted out while minute micro plastic particles were sorted out using a dissection microscope fitted with an ocular lens and finally identified under a light microscope [24]. Micro plastics selected were washed and left to dry at ambient temperature for polymer identification and confirmation using Fourier transformed infra-Red (FTIR) spectroscopy with a single-element MCT detector. Cary 620–670 FTIR microscope, equipped with a GeATR crystal (Agilent Technologies) was used for the analysis and recorded directly with a spectra1 resolution of 8cm⁻¹, in the 650-4000 cm⁻¹ spectral range.

2.5 Biodegradation of Micro Plastic Particles by Gravimetric Method

Micro plastics isolated from the water samples were weighed (initial weight), aseptically transferred to mineral salt broe medium and inoculated with isolates (hydrocarbon utilizing fungi from the estuary) to determine the biodegradability of plastic fragments. Controls were maintained with plastic discs in the medium free of microbe. Fungi removed from the plastic

surfaces were confirmed by microscopic examination. Different flasks were kept in a shaker for forty-five days at room temperature then, the micro plastic particles were collected, washed using de-ionized water, shaded-dry and then final weight was obtained. The percentage degradation (weight loss) was ascertained using the gravimetric method. The formula described by [25] an [26] was used:

$$\text{Weight Loss (in \%)} = \frac{(\text{Initial Weight} - \text{Final Weight})}{\text{Initial Weight}} \times 100$$

2.6 Statistical Analysis

All the experiments were carried out in duplicates (n = 2) and the results are presented in mean values with standard deviation (Mean \pm SD). Duncan multiple range tests, two-way ANOVA and SPSS version 22 were used to check the significance of the data and mean separation.

3. RESULTS AND DISCUSSION

The mean fungal group counts (Cfu/ml) of the surface water during the wet and dry seasons are shown in Fig. 2. The highest mean fungal group counts (4.24×10^4 Cfu/ml) were recorded at station A with the least THF (2.72×10^4 Cfu/ml) recorded at station A during the dry season. Mean hydrocarbon utilizing fungi was highest at station A (1.78×10^4 Cfu/ml) during the wet period while the least HUF (1.46×10^4 Cfu/ml) was recorded at station A during the dry period (Fig. 2). Micro plastic particles such as polyethylene and polypropylene are the two most abundantly produced globally.

Using morphological and microscopic features, a total of eight (8) fungal species were identified from the different sampling stations. The fungal species were identified as *Aspergillus niger*, *Penicillium* spp., *Rhizopus* spp., *Mucor* spp., *Aspergillus nidulans*, *Fusarium* spp., *Microsporium canis*, and *Aspergillus fumigatus*. Microorganisms have been described as ubiquitous and their presence particularly in an aquatic environment relies on the nutrient compound being added during natural storm water runoffs as well as soil erosion [27]. These identified isolates may have entered the Ohiakwu River through runoff or as a result of contamination by human and animal sources. Investigations on microbial loads in the aquatic environment provided a useful basis for assessing the extent of contamination of coastal waters and also the potential health risks to seafood consumers. Microbial communities, however, are usually very complex in their association, thus, very challenging. The Ohiakwu River has been heavily contaminated from human activities [28,29,30] just like every other river in Nigeria. These human activities have affected the quality of the river. The results showed that the fungal load varied during the study period with the wet season having a higher fungal load than the dry season. The incidence of high fungal load in the river provides a useful basis for assessing the extent of contamination of coastal waters as well as the potential health risks posed to seafood consumers. Such magnitude of the composition of the microbial community may not be unconnected with favorable adaptation in the organs, high gut microbiome metabolism and accentuated anthropogenic inputs in the ecosystem [29,30].

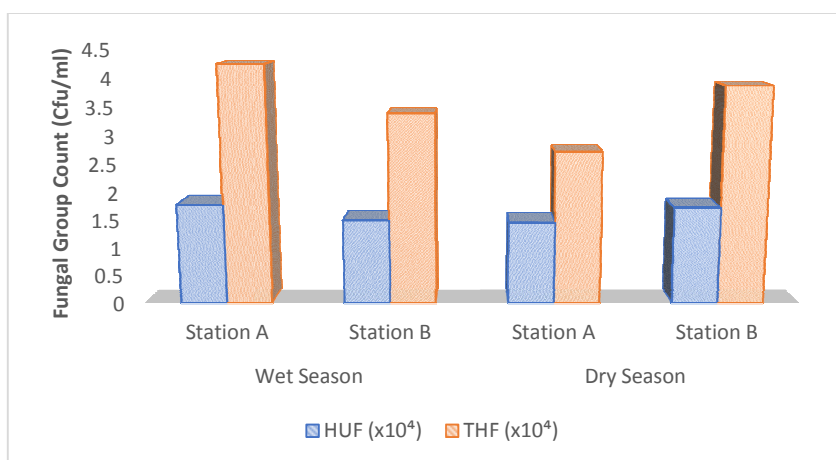


Fig. 2. Mean fungal group counts of surface water

The percentage composition of identified micro plastic particles by mass and abundance in the surface water as depicted by FTIR spectroscopy analysis is shown in Fig. 3. FTIR spectra revealed that the water samples contained micro plastics in the following proportions; 0.01% low density polyethylene (LDPE), 0.11% protein, 0.15% polystyrene, 0.37% polyamide, 1.14% cellulose, 1.21% polyurethane, 1.90% polyvinyl chloride, 3.11% polyester and 92% polypropylene, respectively. Although there are synthetic micro plastic particles in the sample, the most abundant were polypropylene, polyester, polyvinyl chloride, and polyurethane.

The micro plastic particle FTIR spectrum showed the percentage of polypropylene (92%) in the plastic particle with its peaks at a wavelength of 1700 cm^{-1} and 2900 cm^{-1} bands (Fig. 4).

The identified polypropylene particle spectrum obtained using FTIR was compared with a referenced polypropylene particle spectrum. The identified polypropylene particles spectrum (blue thread) matched with the referenced polypropylene particles spectrum (orange thread) with peaks consistent with polypropylene at bands ranging between $2800\text{ cm}^{-1} - 3000\text{ cm}^{-1}$ and 1300 cm^{-1} and 1700 cm^{-1} (Fig. 5).

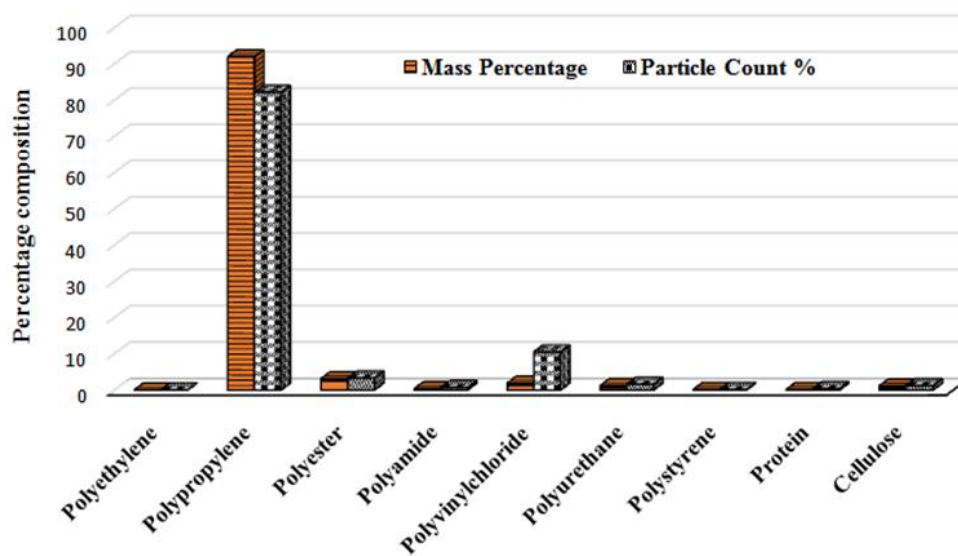


Fig. 3. Percentage composition of different identified plastic particles

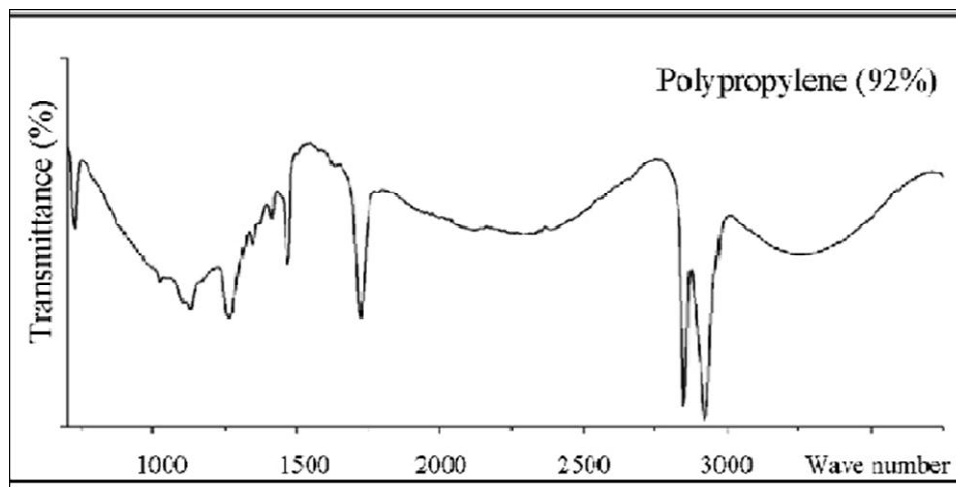


Fig. 4. FTIR spectra of polypropylene particle

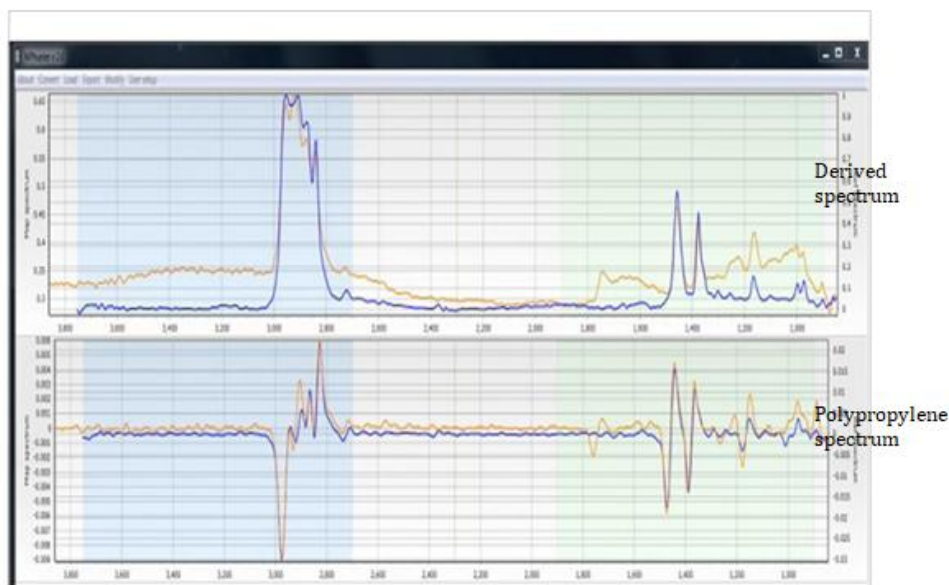


Fig. 5. Referenced polypropylene particle spectrum (blue) with an identified polypropylene particle spectrum (orange)

[29,30] reported similar findings that micro plastic particles are present in aquatic environments. [31] had also reported micro plastic particles at the seafloor of the river. [32] reported that due to the small size of micro plastic particles, which is equivalent to the sizes of plankton which this mullet feeds on, they may be mistaken as food and ingested by aquatic organisms. These findings corroborated the results of this study for the presence of micro plastic particles in the study area.

Micro plastics have myriad applications such as polymer blending processes, food packaging materials, medical, beverage as well as automobile industries and are widely distributed [12,33,34,35]. This could also explain the abundance of polypropylene, polyester, and polyvinyl chloride in the study area. The levels of occurrence of micro plastic (polypropylene) particles and the fungal load may vary due to contamination level, geographical location, differences between micro plastics or microbial community types and the extraction/isolation methodologies and techniques [29,30].

Among the fungal isolates, *Aspergillus fumigatus* and *A. niger* were mostly active in degrading the micro plastic (polypropylene) particles with a mean % weight loss of 71.09% and 53.09%, respectively while the least active was

Penicillium species with a mean % weight loss of 28.64% during the study period (Fig. 6).

According to the findings by [36], majority of plastic degrading fungi are of the genera, *Aspergillus*, *Penicillium*, *Fusarium*, and *Paecilomyces*. The mechanical pressure applied by the fungi during growth and hyphal penetration into the plastic layer, the synchronal secretion of various enzymes and radicals are all characterized as the degradation of plastics by fungi [37]. The mechanism of plastic degradation by microorganisms happened in three steps, in the first step, microorganisms attach to the plastic particles, in the second step, they grow around the particle, and lastly, these microorganisms degraded the particle and used it as a carbon source [29,30].

Exposure of plastic particles to weathering would have enhanced the process of degradation [17,18]. Furthermore, increased ridges on the plastic surface after two weeks indicate microorganism attack. The spectrum of the plastic fraction showed $-C-H$ stretch at $2800 - 3000\text{ cm}^{-1}$ (Alkane), $-C-H$ bend at 1470 cm^{-1} (Alkane), and $-CH_3$ (Methyl) confirmed the hydrocarbon compound structure. Besides, both spectra at $1665 - 1800\text{ cm}^{-1}$ (ketone) and at $1000 - 1200\text{ cm}^{-1}$ (alcohol and ester) validate that certain limited oxy-functionalization of carbon chain had occurred [35,38].

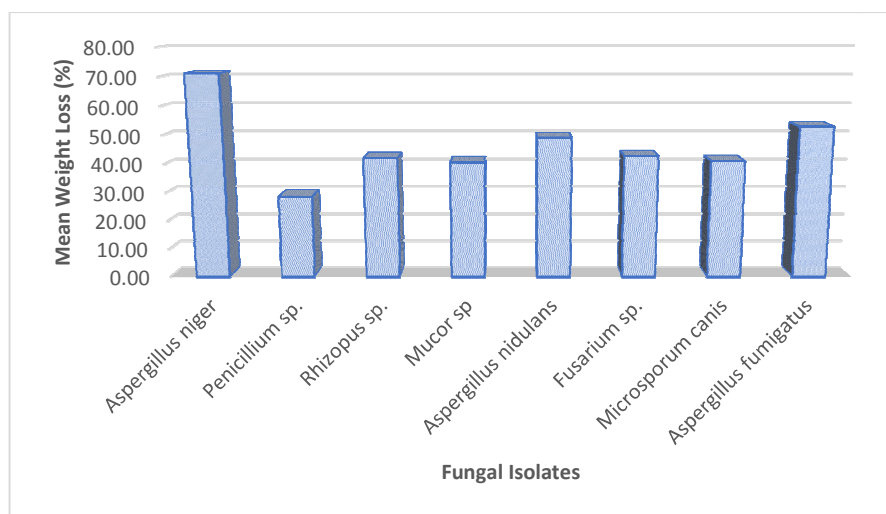


Fig. 6. Degradation of micro plastic particles by fungi

According to the works by [39], fungi could degrade numerous forms of plastics. [40] also reported plastics degradation below laboratory conditions by some species of fungi, *Gliocladium viride*, *Aspergillus awamori*, and *Mortierella subtilissima*. Micro plastic biodegradation in terms of weight loss as a result of biological process occurring in the media and as a result of chemicals within the media has been reported. These microbes first of all adhere to the surface of the microplastic surface that exposes it to microbial colonization of the plastic particles followed by the secretion of extracellular enzymes that bind to the plastic particles and cause hydrolytic cleavage [29,30]. The micro plastic is then degraded into oligomers, monomers and mineralized to carbon dioxide (CO₂) and water (H₂O) utilized by microbes as carbon and energy sources [41]. The three fungal species (*A. niger*, *A. nidulans*, and *A. fumigatus*) could utilize plastic material as carbon and energy sources and possess greater potential to degrade polypropylene polymer. Polymers in their natural state can be degraded to some extent by microbes [29,30]. Microbial degradation of other plastic polymers like polyethylene has been reported by [26] and [42].

4. CONCLUSION

The study has revealed that isolates of fungi from the surface water of the Ohiakwu River could degrade micro plastic (polypropylene). The order of degradation was *Aspergillus niger* > *A.*

fumigatus > *A. nidulans* > *Fusarium* spp. > *Rhizopus* spp. > *Microsporium canis* > *Mucor* spp. > *Penicillium* spp. and their potential in degrading plastics can be utilized as biodegraders of plastic in our surroundings. The leading active fungi with the greatest degrading potentials are *A. niger* (71.1%) and *A. fumigatus* (53.1%), respectively. Therefore, this research is important for industrial development and research purposes. In the natural environment, different kinds of microorganisms play vital roles in numerous steps concerning plastic degradation in general and polypropylene in particular. Studying the working of a consortium of microorganisms together gives insight for future efforts towards plastic degradations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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