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Mycoremediation of Soil Contaminated with Low Density Polyethylene (LDPE) Bags using Fungus (*Pleurotus ostreatus* Jacq. Ex. Fr.)

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Abstract
Mycoremediation of soil contaminated with coloured and transparent low density polyethylene bags using the fungus (*Pleurotus ostreatus* J. Ex. Fr.) were investigated .The fungal spawn was multiplied under laboratory conditions using 78% sawdust, 1% sucrose, 20% wheat bran, 1% calcium and 65% of water. The fungi ramified from this medium were used to investigate its capacity in remediation of soils contaminated with transparent and coloured polyethylene. Equal quantities of the fungi were introduced into similar quantities of coloured and transparent low density polyethylene respectively. Multiplication of fungal spawn lasted for two months while the remediation process lasted for four months. The result showed significant difference ($P<0.05$) in the fixed carbon content of myco-remediated soil compared to the control and that of soil devoid of organic matter. Numerical values were 30.33, 32.78 and 32.53 respectively for soil devoid of organic content, non remediated soil and myco-remediated soil. The cation contents were also significantly better in the mycoremediated soil ($P<0.05$) than that of non remediated soil except for zinc and iron.

Introduction

Large turn- out of domestic organic waste and polyethylene bags in many commercial cities in Nigeria has become serious environmental challenge to human health` and development. Afolabi (2000) reported waste generation of 0.4kg/person/day (19.2 million tons) based on the findings of CASSAD (1997) and a projection of 28 million tons in 2010 when Nigerian population was 151 million. Nigeria population however has been growing at the rate of 3.2% and the more the population growth the more the waste turnout. Of all the waste generated, polyethylene has become a menace due to the usage of the polyethylene packaged sachet water usually referred to as "pure water" all over the country and the use of polyethylene materials in packaging of food products.

Polyethylene is a thermoplastic polymer consisting of long chain monomer produced via polymerization of ethane (Kahovec et al. 2002). According to him, polyethylene are classified into high density polyethylene (HDPE), medium density polyethylene (MDPE) and low density polyethylene (LDPE) based on their solid volume and branching characteristics. LDPE is defined by density range of 0.910 – 0.940g/cm3 and high degree of short and long chain branching. Orhan (2005) estimated polyethylene accumulation in land- fills to be as high as 25 million tons per year, as they remain un-decomposed for over 1000 years. The National Geographic data noted that of the 500 billion polyethylene bags used per year, millions never make it to land- fill, as they are caught up by wind and are found stuck to chain link fences, ensnared on tree branches or clogging drainage channels. Apart from the problem of non degradability of polyethylene bags and associated soil damage ((Shah (2008) and Catherine (2010)), its blockage of drainage ditches and resultant flooding problems (Feroz, 2009), Catherine (2010) also reported that polyethylene has carcinogenic properties. Catherine (2010) also observed that small, non mobile marine organisms (byrozoans, barnacles, polychaete worms', hydroids, crabs and mollusk) colonized on polyethylene materials in such water bodies are carried to new locations where they constitute further environmental problems.

Although polyethylene can be recycled or processed into other useful products, such procedures are costly and the amount recycled is still low compared to the amount produced. Costa (2007) recommended pyrolysis as a counter measure to the environmental threat posed by polyethylene. Adenipekun and Isikhuemhen (2008) listed *Pleurotus ostreatus, Pleurotus tuber-regium and Pleurotus pulmunarius and Lentinus squarrosulus mont* among the mushroom with bioremediation potentials. Degradation of polycyclic aromatic hydrocarbons by *Pleurotus ostreatus* in soils contaminated with crude oil was reported by Adenipekun *et al.* (2011a). This study aims at assessing the potentials of the fungus *Pleurotus ostreatus* in remediation of polyethylene contaminated soil.

Materials and methods

i. Identification of the fungus

The fungi was identified based on the classification key given by (Berbee and Thylor, 1999), and samples of the fungi were confirmed authoritatively by Dr.Adenipekun C.O. of the Botany Department, University of

Ibadan, Oyo State of Nigeria.

ii. Multiplication of fungal spawns

Fungal spawns collected from the Botany Department of University of Ibadan in Oyo state of Nigeria were multiplied based on the method adapted from Fasidi et al. (2008). 780g of sawdust sieved out of the lot collected from Umuokpu timber sawing station in Anambra State, was used for the research after removal of stones and other materials. The sawdust was moistened by soaking in hot water (just about to boil) for one hour and then squeezed between two palms to remove excess water. 200g of wheat bran and 10g of calcium carbonate were added to the saw dust as well as 10g of sucrose mixed in a little quantity of water to provide nutrient for the fungus to be grown in the medium. The mixture was thoroughly mixed with wooden stick and poured into 9 specimen bottles of 12.5 x 4.5 x 2 cm (three quarter filled). Each bottle was plugged with cotton wool and covered on the outside with aluminum foil before being sterilized for 30 minutes in a pressure pot. Thereafter, the bottles were allowed to cool overnight, then each bottle was opened and inoculated with a teaspoonful of mycelium and incubated in a dark room for two months at temperature of 30 + 2° C, during which time the mycelium filled all sides of the bottle.

iii. Pyrolysis of Transparent and Coloured Low Density Polyethylene Materials

The method was self designed, adapted from Costa (2007), Orhan and Buyukgungore (2000) and Shah (2008) but modified to make it practicable for low income citizens. Used "pure water" sachets collected from street corners, drainage channels and refuse dumps represented the transparent low density polyethylene while sachets of biscuits, milk, detergents, nodules, plantain and potato chips made up the coloured low density polyethylene. In each class of low density polyethylene, the bags were cut open, strengthened out and separately washed in lukewarm soapy water and repeatedly washed in running water to ensure removal of impurities especially oil materials, thereafter they were air dried for two days to remove the moisture. The air dried bags were put in separate big sized beverage containers (1000ml) used as improvised furnace, with each lid having a small perforation into which a thermometer with up to 300°C was fitted. Three of the beverage containers labeled A1, A2 and A3 were half filled with dried, washed sandy soil; three other containers labeled B1, B2 and B3 were stuffed with transparent low density polyethylene bags, while the remaining three containers C1, C2 and C3 were stuffed with coloured polyethylene bags. Each of the containers with its content was heated in a separate chamber constructed over a local oven to ensure that the polyethylene bags were heated in an environment with limited oxygen (Costa, 2007). The heating continued until when the polyethylene has fully melted, the temperature was recorded, the thermometer was removed and the perforation fitted with 6 inches nail after the contents of each of the containers in group B and C has been thoroughly mixed with 10kg dried washed sandy soil. Upon cooling, the mixture in each container in group B and C were air dried for 24 hours. The dried, hardened mass in each container was crushed separately in a hammer mill and sieved with a sieve of mesh size 0.2 x 0.9mm to remove the uncrushed particles. The content of each container in group B and C was then inoculated with teaspoonful mycelia from the *Pleurotus ostreatus* culture. The inoculated bottles were incubated in a dark room at temperature of 30 + 2°C for four months to observe the growth fungus in each medium.

iv. Preparation of Sample for Chemical Analysis

At the end of four months incubation period, the mycelium in each bottle was scooped out, placed in a flat ceramic plate and air dried for a whole day and transferred into labeled specimen bottles, before they were taken to Chemistry Department of Projects Development Institute (PRODA, an agency of the Federal Ministry of Science and Technology) in Enugu, Enugu State of Nigeria, where the mineral content of each soil was analysed. The cations (Ca, Zn, Fe, K and P) were analysed using Atomic absorption spectrophotometer (AAS), nitrogen by Ammoniacal Nitrogen Devarda method, and fixed carbon by gravimetric method while the pH was determined using pH meter.

Results

The result of mycoremediation of soils contaminated with coloured low density polyethylene using the fungus *Pleurotus ostreatus* is presented in Table 1, while that of mycoremediation of non – coloured, transparent low density polyethylene materials is shown in Table 2. Table 1 showed significant differences ($P< 0.05$) in the fixed carbon content of the soil samples contaminated with coloured low density polyethylene and that of uncontaminated and unremediated soil as well as that of contaminated but unremediated soil; while the uncontaminated, un-remediated soil had fixed carbon content of 30.33% that of contaminated, unremediated soil was 33.78% but the mycoremediated soil had fixed carbon content of 32.53%. The cation content of the soil samples also differed significantly ($P< 0.05$), Ca, K and Zn content were 0.03, 0.04 and 0.01% for uncontaminated, un-remediated soil, 0.001, 0.02 and 0.05% for contaminated, unremediated and 0.09, 0.05 and 0.02% for mycoremediated soil. Similarly Table 2 showed the result of mycoremediation of soil contaminated with non-coloured, transparent low density polyethylene materials, there was no difference in the fixed carbon content of soils contaminated with either coloured or transparent low density polyethylene materials.

Discussion

The result of this experiment showed the possibility of biodegradation of polyethylene using the fungus *Pleurotus ostreatus*. The reduction in fixed carbon from 32.78 to 32.53 in both the soils contaminated with either transparent or coloured low density polyethylene after four months of incubation with *Pleurotus ostreatus* showed that bioremediation has taken place. The result corroborates the report of Adenikpekun (2008) who listed *Pleurotus ostreatus, Pleurotus tuber-regium and Pleurotus pulmunarius* among the mushroom with bioremediation potentials. The process of pyrolysis followed in this study also agrees with Costa (2007) who stated that heating polyethylene material in the absence of oxygen induced depolymerization to yield radicals and other small molecule of hydrocarbons

Conclusion

The directorate for waste management in Anambra State and Nigeria in general should employ the use of pyrolysis and treatment of polyethylene waste with *Pleurotus ostreatus* as measures to remediate the problem of polyethylene contamination of Nigerian soils.

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Table 1: Mycoremediation of soil contaminated with non-coloured transparent low density polyethylene bags using Pleurotus ostreatus for four months

	Minerals (%)							
Soils	FC	N	P	Ca	K	Zn	Fe	pH
Sandy soil	30.33c	7.50c	0.31	0.03	0.04	0.01	3.10	6.5
Control (washed sandy soil)	32.78a	7.90b	0.10	0.001	0.02	0.05	0.20	6.5
Bioremediated soil	32.53b	8.34a	0.08	0.09	0.50	0.02	Nil	6.5

Table 2: Mycoremediation of soil contaminated with coloured low density polyethylene bags using Pleurotus ostreatus for four months

	Minerals (%)							
Soils	FC	N	P	Ca	K	Zn	Fe	pH
Sandy soil	30.33c	7.50c	0.31	0.03	0.04	0.01	3.10	6.5
Control (washed sandy soil)	32.78a	8.76b	0.001	0.001	nil	0.05	2.20	6.0
Bioremediated soil	32.53b	9.33a	0.05	0.06	0.05	0.01	1.70	6.0